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# FURTHER INVESTIGATIONS ON THE ORIGIN OF TUMORS IN MICE

## III. ON THE PART PLAYED BY INTERNAL SECRETION IN THE SPONTANEOUS DEVELOPMENT OF TUMORS

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In 1907 Loeb showed that it is possible to produce "transitory" uterine tumors in various animals through the coördinated action of an internal secretion of the corpus luteum and of mechanical factors.[1] These new formations, which had the structure of maternal placenta, were described as "deciduomata" or "placentomata," in order to indicate their tumor-like character, and to emphasize the significance of such experiments for the analysis of tumor growth. They were described as "transitory" tumors because retrogressive changes set in after a certain time, and the newly formed tissue finally became necrotic. The possible significance of internal secretions (hormones) in the origin of cancer has been subsequently discussed by Loeb and others (von Hansemann, Isaac Levin, Apolant), though it has not been possible to offer any direct proof of such a relationship.

Extensive statistical data regarding the importance of heredity in the etiology of mouse carcinoma, collected through a period of more than five years, have eventually provided us with the long-desired foundation for more extensive studies on the

[1] Loeb. Leo: Über d. exper. Erzeugung von Knoten von Deciduagewebe, etc., Centralbl. f. allg. Path., 1907, xviii, 563, and a series of later publications.

etiology and the possible prevention of cancer in these animals.[2] The problem to be discussed in the present paper concerns the influence of the corpus luteum on the development of spontaneous mouse-cancer. In order to analyze this influence, we have investigated the frequency of cancer and the age at which it appears in castrated female mice and in those in which breeding had been prevented by segregation of the females. For our purpose it was necessary to choose strains of mice with a well known incidence of cancer and rich in tumors. A certain number of animals from such strains were kept under normal conditions as breeders, others were prevented from breeding, while still others were castrated in our laboratory and then returned to Granby, Massachusetts, so that they might be kept there under the same conditions as the control mice.

The present contribution is intended as the first in which, on the basis of our previous studies on the heredity of cancer in mice, we seek not only to analyze the factors contributing to the origin of cancer, but also to inquire into possible means of preventing the spontaneous development of malignant tumors in mice. Further investigations in this direction have been begun.

## Tumor Incidence and Tumor Age in Castrated Mice

### *Castration of Mice*

#### I. ENGLISH MICE

| *Without tumors* | *With tumors* |
|---|---|
| Group a:[3] 10 mice castrated at about 6 to 7 months of age. | |
| 1 : 11 m. 1 : 14 m. 2 : 19 m. 2 : 21 m. | 1 : 14 m. |
| 3 alive : 23–24 m. | 1 (1 II) |
| 9 (1 I 1 II 7 III) | |

[2] Loeb, Leo: Further observations on the endemic occurrence of carcinoma, etc., Univ. Penn. Med. Bull., 1907–08, xx, 2; Lathrop, A. E. C., and Loeb, Leo: The incidence of cancer in various strains of mice, Proc. Soc. Exper. Biol. and Med., 1913, xi, 34; Loeb, Leo: Some recent results of cancer investigations, Lancet-Clinic, 1913, cx, 664; Lathrop, A. E. C., and Loeb, Leo: Further investigations on the origin of tumors in mice. I. Tumor incidence and tumor age in various strains of mice, Jour. Exper. Med., 1915, xxii, 646.

[3] A figure followed by "m" signifies the age of the mice in months at the time of death; thus, "1 : 11 m." means that one mouse died at the age of eleven months. In case the animals were still alive at the time of the last examination, this fact is expressly stated.

| *Without tumors* | *With tumors* |
|---|---|
| GROUP b: 14 mice castrated at 3 to 6 months. | |
| 3 : 6 to 8 m. 2 : 8 to 10 m. 2 : 16 to 18 m. | 0 |
| 5 : 18 to 20 m. 2 alive : 20 to 22 m. | |
| GROUP c: 13 mice castrated at about 6 months. | |
| 2 : 8 m. 2 : 16 m. 1 : 18 m. 1 : 19 m. | 0 |
| 3 : 23 m. 4 alive : 25 m. | |
| 13 (2 I 3 II 8 III) | |
| GROUP d: 17 mice castrated at 4 to 6 months. | |
| 4 : 18 m. 9 alive : 22 m. | 1 : 13 m. 1 : 15 m. |
| | 1 : 16 m. 1 : 18 m. |
| 13 (4 II 9 III) | 4 (4 II) |
| Total of castrated mice: | |
| 49 (8 I 10 II 31 III) | 5 (5 II) |
| 91% (16% I 20% II 64% III) | 9% (100% II) |

The following additional experiment, concerning only a single mouse, is without much significance. One of a group of English Sable mice (descendants of tumor mouse 437) was castrated at the age of 12 to 13 months. This mouse died at the age of 14 months without a tumor, while the non-castrated controls of this group all died with tumors in the following order: 1 : 8 m.; 2 : 10 m.; 2 : 11 m.; 2 : 14 m.; total = 7 (5 I 2 II) = 100 per cent. The castrated mouse was the only one which died without a tumor at the age of 14 months or below. Altogether, among 55 castrated English mice, 5 developed tumors = 9 per cent. In none of the mice which had tumors did the neoplasm become manifest in the first age period; in all cases it appeared in the second.

To the controls already mentioned in the group of English Sable 437, which had all developed tumors at an age when the only mouse castrated in this group died without tumors, we may add certain data, previously published,[4] relating to English mice and especially to Group A and English Sable, as further control.

[4] Lathrop, A. E. C., and Loeb, Leo: Further investigations on the origin of tumors in mice, I. Tumor incidence and tumor age in various strains of mice, Jour. Exper. Med., 1915, xxii, 646.

| *Without tumors* | *With tumors* |
|---|---|
| English A (Total): | |
| 37% (67½% I 20% II 12½% III) | 63% (47% I 46% II 7% III) |

The various groups did not differ essentially from these figures.

| | |
|---|---|
| English Sable (Total): | |
| 30% (65% I 25% II 10% III) | 70% (66% I 27% II 7% III) |

Here also the figures for the various groups did not differ essentially from the total. Certain variations, of course, occur, especially if the groups considered are smaller. We also wish to add some hitherto unpublished data for English A and English Sable to serve as controls for the castrated English mice.

| | |
|---|---|
| (a) English A Controls: | |
| 1 (1 III) | 9 (1 I 6 II 2 III) |
| (b) English Sable Controls: | |
| 13 (9 I 4 II) | 11 (7 I 3 II 1 III) |
| 54% (69% I 31% II) | 46% (64% I 27% II 9% III) |

While in this case the tumor rate is slightly lower than in the average of the English Sable, yet the age at which the tumors developed is characteristic of the English Sable and of English mice generally (with the exception of English Silver and English Silver Fawn); the tumors appeared very early, a considerable majority in the first age period.

We may conclude, from these data, that if mice are castrated at or below the age of 6 months the tumor incidence will be markedly diminished; we found it decreased from between 60 and 70 per cent in the controls, to 9 per cent in the castrated mice. Furthermore, while the majority of tumors appeared during the first age period in the controls, they were delayed in the castrated mice to the second age period. Castration performed at a period between 4 and 6 months, however, when the mice have already entered the breeding age, does not altogether prevent the development of neoplasms. The smaller number

of tumors among the castrates reduces the mortality, so that a greater number of these mice reach a more advanced age than do the normal mice.

Our conclusions thus far hold good only for mice castrated at an age not above 6 months, for we have only begun to investigate the effect of castration at a higher age. In this second series we have observed the following castrated mice:

| | *Without tumors* | *With tumors* |
|---|---|---|
| (a) [$8\frac{1}{2}$ + English Sable (328)] $F_4$ | | |

Six mice were castrated at 7 to 8 months.

| | |
|---|---|
| 1 : 11 m. 1 alive : 16 m. | 1 : 9 m. 1 : 11 m. 2 : 12 m. |

Of this group, one developed a tumor at the age of 7 months, and another probably had one at the same age, before the group had been used for castration.

(b) [$8\frac{1}{2}$ + English Sable (328)] $F_3$

Seven mice were castrated at the age of about 10 months.

| | |
|---|---|
| 3 : 13 m. | 1 : 12 m. 2 : 13 m. 1 : 14 m. (The last mouse had two tumors.) |

Before this group was used for castration, two had died at the age of 9 months without tumor, and two developed tumors when 10 months old.

Total of mice castrated at an age higher than 6 months:

| | |
|---|---|
| 5 (1 I 4 II) | 8 (5 I 3 II) |
| 38% (20% I 80% II) | 62% (63% I 37% II) |

Similar non-castrated hybrids, $8\frac{1}{2}$ + English Sable (328), mentioned in a previous paper,[5] will serve as controls for the castrated mice.

[5] Lathrop, A. E. C., and Loeb, Leo: Further investigations on the origin of tumors in mice. II. Tumor incidence and tumor age in hybrids, Jour. Exper. Med., 1915, xxii, 713.

| *Without tumors* | *With tumors* |
|---|---|
| 19 (13 I 5 II 1 III) | 28 (19 I 9 II) |
| 40½% (69% I 26% II 5% III) | 59½% (68% I 32% II) |

Since then we have made additional observations on the tumor incidence in these non-castrated hybrids.

| | | |
|---|---|---|
| $F_2$ | 10 (7 I 1 II 2 III)<br>43% (70% I 10% II 20% III) | 13 (7 I 5 II 1 III)<br>57% (55% I 38% II 7% III) |
| $F_3$ | 8 (5 I 3 II)<br>23½% (62½% I 37½% II) | 26 (18 I 7 II 1 III)<br>76½% (69% I 27% II 4% III) |
| $F_4$ | 21 (16 I 5 II)<br>62% (76% I 24% II) | 13 (10 I 3 II)<br>38% (67% I 23% II) |

We have investigated, in addition, a special group of the same hybrids, the offspring of the mother 782a, which was a 328 $F_2$ mouse.

| | |
|---|---|
| 7 (5 I 2 II) | 15 (8 I 7 II) |
| 32% (72% I 28% II) | 68% (53% I 47% II) |

Total of all new controls of these hybrids:

| | |
|---|---|
| 46 (331 11 II 2 III) | 67 (43 I 22 II 2 III) |
| 41% (72% I 24%) | 59% (64% I 33% II 3% III) |

We see in the case of these hybrids again that the figures for cancer rate and cancer age agree very well with the previous figures for the same hybrids. We may furthermore provisionally conclude that castration carried out in mice above the age of 6 months does not have a noticeable influence on cancer rate or cancer age. We are, however, aware of the fact that as yet the number of observed animals in this latter series is rather small, and that further experiments are desirable in order to confirm the provisional interpretation of these figures. We are also aware of the fact that in the second series we did not use the same strain as in the first, and that possibly the effect of castra-

tion may not be equally pronounced in all strains; we intend to investigate this aspect of the problem in further experiments.

In an additional experiment with the strain [English + (8 + German) $F_2$] only 2 mice survived the operation and the long journey a week or two later. These mice, castrated at the age of fourteen months, died two months later without tumors. The uncastrated members of this group showed the following:

| *Without tumors* | *With tumors* |
|---|---|
| 5 (3 I 2 II) | 5 (1 I 4 II) Those dying with tumors in the II age period were all younger than 16 months. |

According to previous records, this strain has a tumor rate of 63 per cent (76 per cent I), the majority of the tumors appearing in the first age period.

## TUMOR INCIDENCE AND TUMOR AGE IN MICE PREVENTED FROM BREEDING

In a previous communication[6] we published data indicating that the cancer incidence is lower and the tumor age higher in mice that are prevented from breeding than they are in breeding mice. Since the difference did not seem to be equally pronounced in other strains, in some of which it was, in fact, only slight, we have subjected the question to a more extended investigation.

### I. ENGLISH

#### (*1*) *English No. A.* (*Non-breeding mice*)

(A) OLD RECORDS

| *Without tumors* | *With tumors* |
|---|---|
| 13 (5 I 6 II 2 III) | 11 (1 I 6 II 4 III) |
| 54% (38% I 46% II 16% III) | 46% (9% I 55% II 36% III) |

(B)

| | |
|---|---|
| 2 (1 I 1 II) | 9 (7 I 2 II) |
| 18% (50% I 50% II) | 82% (77% I 23% II) |

[6] Lathrop, A. E. C., and Loeb, Leo: The influence of pregnancies on the incidence of cancer in mice. Proc. Soc. Exper. Biol. and Med., 1913, xi, 38.

| *Without tumors* | *With tumors* |
|---|---|
| Total: | |
| 15 (6 I 7 II 2 III) | 20 (8 I 8 II 4 III) |
| 43% (40% I 47% II 13% III) | 57% (40% I 40% II 20% III) |
| II + III 43% | II + III 57% |

Not including the animals that died without tumors under the age of 12 months.

| | |
|---|---|
| 9 (7 II 2 III) | |
| 31% (78% II 22% III) | 20 (8 I 8 II 4 III) |
| | 64% (40% I 40% II 20% III) |

*Controls (breeding mice)*[7]

| | |
|---|---|
| Total: | |
| 64 (43 I 13 II 8 III) | 111 (52 I 51 II 8 III) |
| 37% (67½% I 20% II 12½% III) | 63% (47% I 46% II 7% III) |
| II + III 26% | II + III 74% |

Not including those controls that died at or under the age of 12 months without tumors.

| | |
|---|---|
| 21 (13 II 8 III) | 111 (52 I 51 II 8 III) |
| 16% (62% II 38% III) | 84% (47% I 46% II 7% III) |

There has been observed recently a small group of controls which were not included among the old controls.

| | |
|---|---|
| I (1 III) | 9 (1 I 6 II 2 III) |
| 10% (100% III) | 90% (11% I 67% II 22% III) |

In this last group the number of animals is very small.

We see from these records that the tumors were somewhat more frequent, and appeared earlier, in mice that had been breeding than in the non-breeders.

*(2) English 101*

| | |
|---|---|
| (Non-breeding mice) | |
| 18 (2 I 9 II 7 III) | 24 (12 I 9 II 3 III) |
| 43% (11% I 50% II 39% III) | 57% (50% I 37% II 13% III) |
| II + III 57% | II + III 43% |

[7] For more detailed records of the controls, see Lathrop, A. E. C., and Loeb, Leo, Jour. Exper. Med., 1915, xxii, 651.

Not including those mice that died at or under the age of 12 months without tumors.

| *Without tumors* | *With tumors* |
|---|---|
| 16 (9 II 7 III) | 24 (12 I 9 II 3 III) |
| 40% (56% II 44% III) | 60% (50% I 37% II 13% III) |

Controls (breeding mice)

Total:

| | |
|---|---|
| 29 (20 I 9 II) | 70 (46 I 23 II 1 III) |
| 29% (69% I 31% II) | 71% (66% I 33% II 1% III) |
| II + III 28% | II + III 72% |

Not including those controls that died at or under the age of 12 months without tumors.

| | |
|---|---|
| 9 (9 II) | 70 (46 I 23 II 1 III) |
| 11.5% (100% II) | 88.5% (66% I 33% II 1% III) |

In this group we find again that in the non-breeding groups there are fewer tumors, and that the tumors appear later, than in the breeders.

*(3) English Sable*

(Non-breeding mice)

Group I.

| | |
|---|---|
| 17 (41 10 II 3 III) | 20 (5 I 9 II 6 III) |
| 46% (23½% I 59% II 17½% III) | 54% (25% I 45% II 30% III) |

Group II.

| | |
|---|---|
| 18 (3 I 8 II 7 III) | 18 (2 I 10 II 6 III) |
| 50% (16½% I 44½ II 39% III) | 50% (11% I 55½% II 34½% III) |

Total:

| | |
|---|---|
| 35 (7 I 18 II 10 III) | 38 (7 I 19 II 12 III) |
| 48% (20% I 51½% II 28½% III) | 52% (18½% I 50% II 31½% III) |
| II + III 47½% | II + III 52½% |

Excluding those animals that died in the first period of life without tumors:

| *Without tumors* | *With tumors* |
|---|---|
| 28 (18 II 10 III) | 38 (7 I 19 II 12 III) |
| 42½% (64% II 36% III) | 57½% (18½% I 50% II 31½% III) |

Controls (breeding mice)

Total of English Sable[8]

| | |
|---|---|
| 76 (49 I 19 II 8 III) | 176 (115 I 48 II 13 III) |
| 30% (65% I 25% II 10% III) | 70% (66% I 27% II 7% III) |
| II + III 31% | II + III 69% |

Excluding those animals that died in the first period of life without tumors:

| | |
|---|---|
| 27 (19 II 8 III) | 176 (115 I 48 II 13 III) |
| 13% (71% 29%) | 87% (66% I 27% II 7% III) |

Recently a small additional group of breeding English Sable has been observed.

| | |
|---|---|
| 13 (9 I 4 II) | 11 (7 I 3 II 1 III) |
| 54% (69% I 31% II) | 46% (64% I 27% II 9% III) |

While in this small group the tumor incidence is very slightly below that of the non-breeders, the tumors appear here considerably earlier than in the non-breeders.

In the English Sable, the tumor incidence is higher and the tumor age lower in the breeders than in the non-breeders.

If we compare the total of all the English non-breeding groups with the total of all the English breeders, we obtain the following figures:

(a) Non-breeders:

| | |
|---|---|
| 68 (15 I 34 II 19 III) | 82 (27 I 36 II 19 III) |
| 45⅓% (22% I 50% II 28% III) | 54⅔% (33% I 44% II 33% III) |
| II + III 44% | II + III 56% |

[8] For detailed figures, see previous paper, Jour. Exper. Med., 1915, xxii, 653 and 654.

Excluding mice dying in the first period of life without tumors:

| *Without tumors* | *With tumors* |
|---|---|
| 39% | 61% |

(b) Breeders:

| | |
|---|---|
| 183 (121 I 45 II 17 III) | 377 (221 I 131 II 25 III) |
| $32\frac{2}{3}$% (66% I 25% II 9% III) | $67\frac{1}{3}$% ($58\frac{1}{3}$% I $34\frac{2}{3}$% II 7% III) |
| II + III $28\frac{1}{2}$% | II + III $71\frac{1}{2}$% |

Excluding mice dying in the first period of life without tumors:

| | |
|---|---|
| 14% | 86% |

The consideration of the total of English mice, breeders and non-breeders, confirms the conclusion arrived at previously in considering the individual groups.

II. NO. 8

(Non-breeding mice)

| | |
|---|---|
| $F_4$ 18 (1 I 8 II 9 III) | 1 (1 III) |
| $F_5$ 61 (12 I 28 II 21 III) | 0 |
| $F_7$ 37 (3 I 16 II 18 III) | 2 (1 II 1 III) |
| $F_8$ 20 (20 III) | 2 (2 III) |
| $F_9$ 20 (1I 9 II 10 III) | 0 |
| $F_{10}$ 7 (1 I 6 III) | I (1 III) |

Total:

| | |
|---|---|
| 163 (18 I 61 II 84 III) | 6 (1 II 5 III) |
| 96.5% (11% I 37% II 52% III) | 3.5% (17% II 83% III) |
| II + III 96% | 4% |

Controls (breeding mice)[9]

| | |
|---|---|
| $F_2$ 1 (1 III) | 4 (4 III) |
| $F_3$ 14 (2 I 3 II 9 unknown age) | 8 (3 I 2 II 3 III) |

[9] In the No. 8 strain an error occurred in our previous paper (Jour. Exper. Med., 1915, xxii, 658). Mice belonging to the tenth and eleventh generation were erroneously designated as $F_8$. We give here a corrected list of the No. 8 strain and its generations with the addition of some more recent records.

| | *Without tumors* | *With tumors* |
|---|---|---|
| $F_4$ | 24 (22 I 1 II 1 III) | 11 (3 I 6 II 2 III) |
| $F_5$ | 18 (9 I 9 III) | 9 (4 I 2 II 3 III) |
| $F_6$ | 39 (8 I 15 II 16 III) | 17 (7 I 8 II 2 III) |
| $F_7$ | 29 (19 I 8 II 2 III) | 3 (1 II 2 III) |
| $F_9$ | 12 (7 I 3 II 2 III) | 4 (1 II 3 III) |
| $F_{10}$ | 22 (4 I 3 II 15 III) | 10 (4 II 6 III) |
| $F_{11}$ | 24 (7 I 11 II 6 III) | 1 (1 III) |
| $F_{12}$ | 10 (2 I 3 II 5 III) | 1 (1 III) |
| $F_{14}$ | 1 (1 I) | |

Total (not including offspring of tumor mouse No. 100):

| | |
|---|---|
| 194 (81 I 47 II 57 III 9 unknown age) | 68 (17 I 24 II 27 III) |
| 74% (42% I 24% II 34% III) | 26% (25% I 35% II 40% III) |
| II + III 67% | II + III 33% |

In the eleventh and twelfth generation there is a decrease in the cancer incidence; whether this is accidental or not, we cannot state definitely at present.

If we compare cancer age and cancer incidence in the non-breeding mice, and in all the breeding mice of No. 8, we find a great difference. The cancer incidence is considerably greater, and the cancer age lower, in the breeding mice. It might, however, be more correct to use for comparison only those generations of the breeding mice which correspond to the generations of the non-breeding mice, namely, $F_4$ $F_5$ $F_7$ $F_8$ $F_9$ $F_{10}$. We have, however, to omit $F_8$ in the list of the breeders, as no records of this generation are available.

The total of tumor incidence and tumor age of those generations of the breeding mice is as follows:

| | |
|---|---|
| 105 (61 I 15 II 29 III) | 37 (7 I 14 II 16 III) |
| 74% (58% I 14% II 28% III) | 26% (19% I 38% II 43% III) |
| II + III 34% | II + III 66% |

These figures show likewise a smaller incidence of cancer and a higher tumor age in non-breeding mice.

III. NO. $8\frac{1}{2}$

| *Without tumors* | *With tumors* |
|---|---|
| (Non-breeding mice) | |
| 21 (8 II 13 III) | 1 (1 III) |
| 20 (1 I 4 II 15 III) | 5 (2 II 3 III) |
| Total: | |
| 41 (1 I 12 II 28 III) | 6 (2 II 4 III) |
| 87% (2% I 29% II 69% III) | 13% (33% II 67% III) |
| II + III 87% | II + III 13% |

Not including mice that died in the first age period without tumors:

| | |
|---|---|
| 87% | 13% |

Controls (breeding mice)

(a) Old controls corresponding to the non-breeding mice.

| | |
|---|---|
| 106 (30 I 49 II 27 III) | 25 (6 I 15 II 4 III) |
| 87% (28% I 46% II 26% III) | 19% (24% I 60% II 16% III) |

Total of controls (breeding mice of No. $8\frac{1}{2}$ strain)

| | |
|---|---|
| 131 (43 I 52 II 36 III) | 27 (6 I 17 II 4 III) |
| 83% (33% I 40% II 27% III) | 17% (22% I 63% II 15% III) |
| II + III 81% | II + III 19% |

Not including mice that died in the first age period without tumors.

| | |
|---|---|
| 75% | 25% |

In this strain the tumor incidence is somewhat lower and the tumor age higher in the non-breeding, as compared with the breeding mice.

IV. CARTER

| | |
|---|---|
| (Non-breeding mice) | |
| $F_2$ 22 (31 1 II 18 III) | 0 |
| $F_3$ 21 (1 I 7 II 13 III) | 2 (2 III) |
| Total: | |
| 43 (4 I 8 II 31 III) | 2 (2 III) |
| 96% (9% I 19% II 72% III) | 4% (100% III) |
| II+III 95% | II+III 5% |

Excluding those mice that died in the first age period without tumors:

| *Without tumors* | *With tumors* |
|---|---|
| 95% (21% II 79% III) | 5% (100% III) |
| Controls (breeding mice) | |
| 41 (15 I 14 II 12 III) | 26 (12 I 9 II 5 III) |
| 61% (36% I 35% II 29% III) | 39% (46% I 35% II 19% III) |
| II+III 65% | II III 35% |

Excluding those mice that died in the first age period without tumors:

| | |
|---|---|
| 50% (54% II 46% III) | 50% (46% I 35% II 19% III) |

Here again the tumor incidence is higher, and the tumors appear earlier in the breeding mice than in the non-breeding. In this strain, the difference between the two classes of mice is very marked.

## V. EARLY STRAINS

In addition to the strains upon which we have now reported, we have investigated non-breeding mice in some earlier strains in which the tumor incidence of the breeding mice was only very incompletely established. And inasmuch as the data, notwithstanding their incompleteness and their lack of accuracy, seem to confirm the other observations, we shall briefly mention the figures obtained.

### (*a*) *No. 5*

| *Without tumors* | *With tumors* |
|---|---|
| (Non-breeding mice) | |
| $F_4$ 15 (6 II 9 III) | 0 |
| $F_5$ 11 (4 I 3 II 4 III) | 0 |
| Total: | |
| 26 (4 I 9 II 13 III) | 0 |
| 100% (16% I 34% II 50% III) | 0 |
| Breeding controls | |
| 17 | 9 |
| 65% | 35% |

(*b*) *No. 6*

| *Without tumors* | *With tumors* |
|---|---|
| (Non-breeding mice) | |
| $F_5$ 23 (3 I 10 II 10 III) | 0 |
| 100% (13% I 43.5% II 43.5% III) | 0 |
| Breeding controls | |
| 15 | 8 |
| 65% | 35% |

(*c*) *Family G*

| | |
|---|---|
| 69 (6 I 14 II 49 III) | 7 (1 II 6 III) |
| 91% (9% I 20% II 71% III) | 9% (14% II 86% III) |

The records of the breeding mice in this group are incomplete, but there are records of 16 tumors among 150 breeding mice (9.6 per cent). We may assume that not all tumors were recorded in the breeding mice and that here the cancer incidence was probably higher.

## A COMPARISON OF THE TUMOR AGE OF NON-BREEDING AND OF BREEDING MICE

It is of interest to calculate the tumor age of the non-breeding mice in a similar manner as we did that of the breeding mice in a former publication.[16]

Non-breeding mice:

*English total*

I Period: 150 mice 27 tumors = 18%
I : II + III = 1 : 4 I : II = 1 : 1.3
II Period: 108 mice 36 tumors = 23%
II : III = 1 : 2.2 I + II : III 1 : 1.2
III Period: 38 mice 19 tumors = 50%

Control breeding English mice: Total I : II + III 1 : 3.2 I : II 1 : 1.7 II : III 1.3 : 1 I + II : III 2 : 1. The table shows that the tumor age is higher in the non-breeding mice.

[10] Lathrop, A. E. C., and Loeb, Leo: Jour. Exper. Med., 1915, xxii, 646.

No. 8

(Non-breeding mice)

I Period: 169 mice 0 tumors
I : II + III = 0 : 6 I : II = 0 : $\frac{2}{3}$

II Period: 151 mice 1 tumor
II : III = 1 : 8 I + II : III = 1 : 8

III Period: 89 mice 5 tumors

Control; breeding No. 8: I : II + III = 1 : 9.3 I : II = 1 : 4.7 II : III 1 : 1 I + II : III = 1.2 : 1

Here, also, the tumor age is distinctly higher.

No. $8\frac{1}{2}$

(Non-breeding mice)

I Period: 47 mice 0 tumors = 0%
I : II + III = 0 : 16 I : II = 0 : 4 II : III = 1 : 3

II Period: 46 mice 2 tumors = 4%
I + II : III = 1 : 3

III Period: 32 mice 4 tumors = 12%

Control, breeding No. $8\frac{1}{2}$: I : II + III 1 : 6.2 I : II = 1 : 37 II : III = 1.5 : 1 I + II : III = 1.9 : 1

The tumor age is distinctly higher.

Carter

(Non-breeding mice)

I Period: 45 mice 0 tumors = 0%

II Period: 41 mice 0 tumors = 0%

III Period: 31 mice 2 tumors = $6\frac{1}{2}$%

Control. breeding Carter: I : II + III = 1 : 3 I : II = 1 : 1.3 II : III = 1 : 1.2 I + II : III = 1.4 : 1

Tumors appeared in this case only in the III period. The tumor age is higher.

If we compare the age of the breeding and non-breeding individuals of each strain, we find that the non-breeding mice reach a higher age in each. This is in part due to the lower tumor incidence in the latter; but non-breeding mice which do not develop tumors reach a considerably higher age period than do breeding mice in which neoplasms do not appear, so that generative activity seems distinctly to shorten the life of female

mice in all the strains investigated. The non-breeding mice are usually restless between the ages of 3 and 6 months, though later they become quiet; at about 9 months they become fat, and at 15 months almost all are excessively fat. Only a few of the breeders become fat, and this change is usually associated with a decline in the breeding activity.

The castrated English female mice differ noticeably from ordinary English and especially from the non-breeding English, in being very much wilder and more timid. The non-breeding mice are very tame.

We may conclude that breeding increases the tumor incidence in mice and makes the tumors appear at a lower age. Non-breeding mice reach a higher age than breeding mice; the effect of non-breeding, however, so far as we can state at the present time, is much less marked than the effect of castration done below the age of 6 months. At present it can be said only that the effects of non-breeding, while not great, are nevertheless distinct; they are so constant, however, that they can hardly be referred to accident. The experiments are being continued, and a subsequent report will state whether or not the later experiments confirm the present conclusions.

## INTERPRETATION OF RESULTS

If we now inquire how extirpation of the ovaries influences the development of carcinoma in mice, we have to consider the relation of the ovaries to the mammary gland. It seems an established fact that a substance given off by the corpus luteum induces the periodic growth of the mammary gland during pregnancy, and in some species also during periods of the sexual cycle unaccompanied by pregnancy. It is, therefore, most probable that castration is effective because it eliminates the corpora lutea. We have, furthermore, to consider the fact that the mice subjected to castration were already in the breeding age, and that their mammary glands were, therefore, no longer in an immature condition. Extirpation of the ovaries is in all probability effective because it eliminates the periodic growth of the mammary gland and allows it to remain in an uninterrupted state of rest. But it appears that after the mammary

gland has been for a longer period of life under the influence of the corpus luteum, the threshold of growth processes which allows transition into a carcinomatous condition has been reached; hence, extirpation at later periods of life is found to be without effect.

In accordance with this interpretation of the facts, it is quite clear that prevention of breeding must have less effect than castration. Prevention of breeding does not eliminate the periodic appearance of corpora lutea, though the corpora lutea in the ordinary sexual cycle remain smaller than those developing during pregnancy, and their duration of life is shorter. We find, therefore, that in non-breeding animals the tumor incidence is somewhat diminished and the tumor age increased.[11] The nursing of their young may be an additional factor which favors the development of cancer in the breeders.

It is quite clear that hereditary differences in the tumor rates of different strains are very much greater than could be explained by the differences in breeding activity of different strains. Moreover, the strain "cream," which has a low tumor rate and is at the same time a strain of poorly breeding mice, has recently much improved in breeding energy without an accompanying increase in the tumor rate. The hereditary factor is, therefore, not identical with that determining the influence of the corpus luteum on the mammary gland, but must have its place of attack elsewhere.

These results demonstrate experimentally for the first time the significance of an internal secretion, or hormone, for the spontaneous development of cancer. It is very probable that mammary cancer is not an exception, and that other substances favoring growth are a factor in the development of carcinoma at other sites. In other tissues, long continued external irritation, and in still others a combination of both factors, may, perhaps, exert a similar effect. It seems probable that any factor which periodically or over long periods of time induces increments in growth energy, may be a factor in the development of carcinoma.

[11] We have already begun a new series of experiments in order to diminish still further the probability of accidental factors entering into our results.

SUMMARY

1. Castration of female mice below the age of six months leads to a very marked decrease in the cancer incidence of these animals, although we have not so far succeeded in preventing cancer altogether under these conditions. The cancer age is increased in castrated female mice. Castration in mice above the age of six months has so far been without effect.

2. The prevention of breeding in female mice decreases the cancer incidence and increases the cancer age, though to a much smaller degree than does castration. Non-breeding female mice reach a higher age than breeders.

3. These results are interpreted as due to the influence of the corpus luteum on the growth of the mammary gland, and as the first experimental demonstration of internal secretion as an etiological factor in the spontaneous development of cancer. The chemical action is superimposed upon a hereditary factor distinct from the former.

# THE MORTALITY FROM CANCER IN THE WESTERN HEMISPHERE[1]

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## INTRODUCTION

The progress of society is largely measured by the more or less effective prevention of poverty, sickness, and premature death. Within a single generation a veritable revolution has been achieved in the control of many important diseases, and some of the most destructive plagues of mankind have been successfully eliminated, or materially reduced in frequency, in the principal civilized countries of the world. Foremost, in the order of importance, are the diseases attributable to infection or contagion, and in many important countries plague, cholera, yellow fever, leprosy, smallpox, typhoid fever, and tuberculosis, as well as most of the acute infectious diseases of infancy, are now measurably and effectively under sanitary control. By way of illustration, attention may be directed to the balance of mortality for four large American cities for which the data are available in a fairly trustworthy form for the last fifty years. The cities considered are New York, Boston, Philadelphia, and New Orleans, and the data may be relied upon as fairly representative for at least the urban centers of population of the country at large.

## DECLINE IN THE DEATH RATE

In the four cities under consideration Asiatic cholera and yellow fever have been practically eliminated. Smallpox has been

[1] Read at the Second Pan-American Scientific Congress, Washington, D. C., January 7, 1916.

reduced from a former rate of 39.5 to a present rate of only 2.4 per 100,000 of population. Scarlet fever, diphtheria and croup, typhoid fever, and pulmonary tuberculosis, as well as the diseases of the stomach and intestines, have materially decreased during the last quarter-century, compared with the twenty-five years preceding the year 1889. The most significant exception to a general decline in the death rate is the increasing mortality from

*Balance of mortality for New York, Boston, Philadelphia, and New Orleans, 1864–1888—1889–1913*

(Rates per 100,000 of population)

| | 1864-1888 | | 1889-1913 | |
|---|---|---|---|---|
| | Deaths | Rates | Deaths | Rates |
| Smallpox | 23,799 | 39.5 | 3,308 | 2.4 |
| Asiatic cholera | 4,506 | 7.5 | 10 | 0.01 |
| Yellow fever | 8,469 | 14.0 | 821 | 0.6 |
| Scarlet fever | 39,983 | 66.3 | 25,560 | 18.8 |
| Diphtheria and croup | 74,274 | 123.2 | 79,396 | 58.3 |
| Typhoid and typhus fevers | 32,042 | 53.1 | 33,573 | 24.7 |
| Pulmonary tuberculosis | 220,048 | 364.9 | 303,862 | 223.3 |
| Pneumonia | 113,712 | 188.5 | 315,648 | 232.0 |
| Stomach and intestinal diseases | 164,598 | 298.6 | 266,991 | 196.2 |
| Heart diseases | 62,565 | 103.7 | 223,991 | 164.6 |
| Nephritis | 47,479 | 78.7 | 179,258 | 131.7 |
| Cancer | 27,305 | 46.4 | 98,085 | 72.1 |

cancer. In the four cities under consideration the cancer death rate has *increased* from 46.4 to 72.1 per 100,000 of population. In other words, there has been an actual increase in the rate of 25.7 per 100,000 of population, equivalent to a quarter-century increase in the rate of 55.4 per cent.

## INCREASE IN CANCER

In the registration area of the United States, during the decade ending with 1912, the death rate from cancer was 72.8 per 100,000 of population, or 55.7 for males and 90.6 for females. In the registration area, which now includes about two-thirds of the entire American population, the cancer death rate has increased

from 62.9 per 100,000 of population during 1900 to 78.9 during 1913. On the basis of a conservative estimate the annual number of deaths from cancer in the entire United States is approximately 80,000. It is, therefore, not going too far to speak of cancer as a *menace* to civilization and as a disease of the very first order of present-day importance in medicine, surgery, and public health.

## PROBLEMS OF CANCER CONTROL

Largely upon the basis of the statistical evidence regarding cancer frequency and cancer increase throughout practically the entire civilized world, a movement was inaugurated within very recent years effectively to arouse public interest in the possibilities of cancer prevention and cancer control. Chiefly under the direction of the American Society for the Control of Cancer, but in hearty coöperation with national, state, and local medical and surgical societies, a campaign of education has been carried on through the entire country, which, in the opinion of those best qualified to judge, has been decidedly beneficial and warrants favorable anticipations regarding ultimate results. Under the auspices of the American Society for the Control of Cancer, the American Medical Association, medical societies, state and municipal boards of health, etc., and several of the large American life insurance companies, a considerable amount of well considered and instructive information on the cancer problem in its relation to the individual has been published and made available to many who otherwise could not have been reached so effectively and in so brief a period of time. Since the appeal to the public rests largely upon the statistical facts of the cancer situation, conservatively determined by a qualified analysis of the available information, it has seemed advisable to present on this important occasion, a plea for the general acceptance of the principles and methods of the American Society for the Control of Cancer, at least throughout the countries of the western hemisphere.

## GEOGRAPHICAL INCIDENCE

The frequency of cancer, geographically considered, varies enormously throughout the world, but the mortality rate is also decidedly affected by sex, age, race, and marital condition. Apparently less important factors are climate and occupation. For many important countries no trustworthy data are at present available and for most of the states of the western hemisphere the acceptable returns are limited to large cities. When all the available statistics for the civilized world are combined, it appears that during recent years (1908–1912) the average cancer death rate was 65.7 per 100,000 of population for the western hemisphere sphere, which compares with a rate of 72.8 for the eastern hemisphere. In the western hemisphere the country of most importance, whether considered by area or population, is naturally the United States, but even for this country there are at present trustworthy mortality data for only two-thirds of the population, and 40 per cent of the total area. It is, therefore, not feasible to make a thoroughly satisfactory presentation of the facts of geographical incidence for the entire western hemisphere, but the available amount of information is sufficient for the present purpose. Preliminary, however, to a discussion of the variations in the cancer death rate throughout the western hemisphere, certain elmentary statistical considerations, based largely upon the cancer mortality statistics of the United States registration area, are entitled to presentation.

## AGE AND SEX

The table following exhibits the mortality from cancer by age and sex in the United States registration area for the decade 1903–1912.

According to this table the death rate is practically the same for both sexes at ages under 5, a trifle higher for males at ages 5–14, but at ages 15–44 the male rate is 13.6 per 100,000 of population and the female rate 32.1; or, in other words, the female rate is 18.5 per 100,000 of population, or 136.0 per cent, in excess of the male rate. At ages 45 and over the male rate is

*Mortality from cancer, by age and sex, United States registration area, 1903–1912*
(Rates per 100,000 of population)

| AGES | MALES | FEMALES |
|---|---|---|
| | Rates | Rates |
| Under 5 | 3.3 | 3.0 |
| 5–14 | 1.7 | 1.3 |
| 15–44 | 13.6 | 32.1 |
| 45 and over | 236.5 | 366.4 |

236.5, against 366.4 for females; or, in other words, the cancer death rate of females at this age period is 129.9 per 100,000 of population, or 54.9 per cent, in excess of the male rate. This excess in the adult cancer death rate of women is largely, in the United States at least, due to cancer frequency in organs or parts peculiar to the female sex. The table on the following page exhibits the cancer mortality by organs and parts, in the United States registration area, for the period 1903–1912, with an estimate of the number of deaths from cancer for the different organs and parts, according to sex, for the year 1915.

## ORGANS AND PARTS

According to this table the rate of mortality from cancer of the stomach and liver is almost the same for both sexes, but there is a decided excess in the case of females in the mortality rate from cancer of the peritoneum, intestines and rectum. The rate of mortality from cancer of the skin, and of the buccal cavity, is higher among men than among women, but the actual number of deaths from this group of malignant diseases is not of so much importance. The excess in the female cancer mortality is to be found chiefly in the deaths from cancer of the generative organs and of the female breast, accounting for 39.3 per cent of the total mortality from cancer among women, and 35.6 per 100,000 of population of females separately considered. Eliminating cancer of the generative organs and of the breast, it is shown that the cancer death rate for males was 55.7 per 100,000 of population, against 55.0 for females; or, in other words, the actual differences in the rates were relatively of small significance.

As will subsequently be shown, these differences in the incidence of cancer by sex vary widely throughout the world, and it is in the direction of geographical pathology that the outlook is most encouraging that future statistical research will add materially to our understanding of the conditions and circumstances accountable for a high or low cancer death rate.

*Mortality from cancer, by organs or parts, United States registration area, 1903–1912, and estimated cancer mortality of the Continental United States, 1915*

(Rates per 100,000 of population)

| | NO. OF DEATHS 1903–1912 | PER CENT | RATES | ESTIMATED NO. OF DEATHS, 1915* | PER CENT |
|---|---|---|---|---|---|
| MALES | | | | | |
| Buccal cavity | 9,652 | 7.4 | 4.2 | 2,608 | 8.1 |
| Stomach and liver | 64,049 | 49.4 | 27.5 | 15,622 | 48.6 |
| Peritoneum, intestines and rectum | 16,615 | 12.8 | 7.1 | 4,503 | 14.0 |
| Skin | 7,722 | 5.9 | 3.3 | 1,808 | 5.6 |
| Other or not specified organs | 31,746 | 24.5 | 13.6 | 7,577 | 23.6 |
| Ages 45 and over | 111,884 | 86.2 | 236.5 | 28,135 | 87.6 |
| Total all ages | 129,784 | 100.0 | 55.7 | 32,118 | 100.0 |
| FEMALES | | | | | |
| Buccal cavity | 2,163 | 1.1 | 1.0 | 541 | 1.1 |
| Stomach and liver | 64,685 | 31.9 | 29.0 | 16,050 | 33.5 |
| Peritoneum, intestines and rectum | 23,137 | 11.4 | 10.3 | 6,110 | 12.8 |
| Generative organs | 49,747 | 24.6 | 22.3 | 12,348 | 25.8 |
| Breast | 29,685 | 14.7 | 13.3 | 7,235 | 15.1 |
| Skin | 4,306 | 2.1 | 1.9 | 958 | 2.0 |
| Other or not specified organs | 28,698 | 14.2 | 12.8 | 4,640 | 9.7 |
| Ages 45 and over | 164,673 | 81.4 | 366.4 | 39,456 | 82.4 |
| Total all ages | 202,421 | 100.0 | 90.6 | 47,882 | 100.0 |

* Estimated on the basis of the percentage distribution of the mortality from cancer in the registration area in 1913.

Figure 1 graphically illustrates the data of the preceding tables.

## URBAN AND RURAL

In the registration states of the United States during the decade ending with 1912 the urban mortality rate from cancer

# Cancer Mortality — United States Registration Area

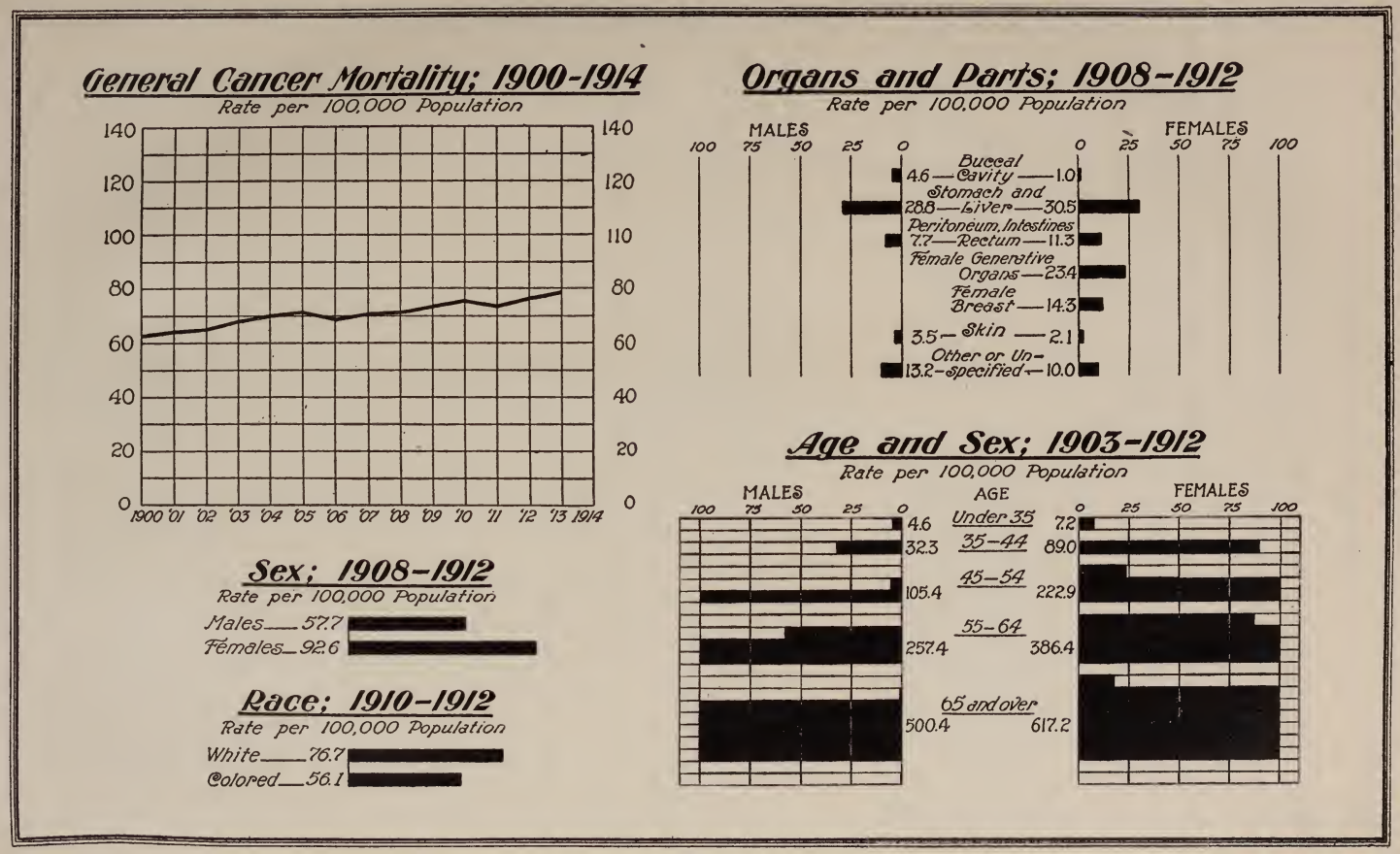


FIGURE 1

was 79.2, against a rural rate of 66.7, per 100,000 of population. The urban rate is partly affected by cancer deaths in hospitals and special institutions for cancer treatment, but when due allowance is made for this factor there would seem to be no reason to question the conclusion that cancer is relatively more common among the American city population, when compared with the corresponding population of the country. Important exceptions, however, are met with in different countries of the world, and the present conclusion applies only to the registration states of the United States registration area. Crude death rates in this respect are quite likely to be misleading in that the proportion of aged persons is almost everywhere larger in the country districts than in the cities.

### RACE

During the period 1906–1912, in thirty large southern cities of the United States the cancer mortality rate of the white population was 80.3 per 100,000 of population, and of the colored population, 55.2. All the available statistics for the United States and other countries emphatically sustain the conclusion that cancer, generally considered, is relatively rare among primitive races. In the case of the American negro, for more than a century and a half in close contact with the white race, and with habits of life closely conforming to those of the Caucasians, there has been a gradual approach in the cancer death rate towards the rate common to the white population, and for cancer of the uterus the rate for the negro population of at least certain American cities is now in excess of the corresponding rate for white women. The lesser liability to cancer among primitive races would, therefore, seem to be rather attributable to habits of life or the mode of living than to racial immunity. Of all the statistical elements of the cancer problem the racial aspect of the disease is extremely interesting and most likely to prove of the first order of practical importance. Several careful investigations have been made in the United States with reference to the occurrence or relative frequency of malignant diseases among North American Indians and the Eskimos of Alaska and Labra-

dor, but without exception the results have been negative. In other words, there are no reasons for questioning the statement that cancer is actually as well as apparently very rare among North American Indians and Eskimos, and that the explanation for the infrequency of the disease is, most likely, that it is due to habits or mode of life rather than to racial immunity.

## LATITUDE AND CLIMATE

The possible correlation of latitude and equivalent climatic conditions to cancer frequency is shown in the table following, illustrating the mortality from cancer in one hundred and thirty cities, according to latitude, for the eastern and western hemispheres, for the period 1908–1912:

*Mortality from cancer in cities, according to latitude, Eastern and Western Hemispheres, 1908–1912*

| DEGREE OF LATITUDE | EASTERN HEMISPHERE | | | WESTERN HEMISPHERE | | |
|---|---|---|---|---|---|---|
| | No. of Cities | Rates per 100,000 Population | Index Number | No. of Cities | Rates per 100,000 Population | Index Number |
| 60 N.–50 N. | 35 | 105.7 | 98 | | | |
| 50 N.–40 N. | 22 | 108.4 | 100 | 26 | 77.3 | 100 |
| 40 N.–30 N. | 6 | 66.9 | 62 | 18 | 85.5 | 111 |
| 30 N.–10 N. | 3 | 13.6 | 13 | 4 | 77.2 | 100 |
| 10 N.–10 S. | 1 | 11.6 | 11 | 3 | 82.7 | 107 |
| 10 S.–30 S. | 1 | 34.4 | 32 | 6 | 38.2 | 49 |
| 30 S.–40 S. | 1 | 90.1 | 83 | 4 | 89.8 | 116 |
| Total | 69 | 98.3 | | 61 | 78.0 | |

According to this table there is no precise degree of correlation, partly, no doubt, because of the fact that the data for tropical countries are too insufficient, and that such cities as Calcutta, Hongkong, and Singapore are not strictly comparable with cities like New Orleans, Havana, and Paramaribo. Furthermore, 60° to 40° north latitude in European countries corresponds rather to 50° to 40° north latitude in the western hemisphere, as regards climatological conditions. It has not been feasible for the present purpose to establish, on the basis of a sufficient

amount of statistical information, the precise correlation of temperature, rainfall, and humidity to cancer frequency, but the suggestion may be made here that such a study would add a useful contribution to cancer knowledge.

### HABITS AND ECONOMIC CONDITIONS

The question may also be raised as to whether the observed differences in the relative frequency of cancer between civilized and primitive man are not primarily conditioned by habits of life, chiefly, of course, diet, which is largely a matter of material well-being. Native races are generally underfed, at least with regard to what in civilized countries is normally considered a nutritious diet, often, however, erroneously confused with an excess in food consumption. At least in the case of the native American Indians it is a safe assumption that the large majority are underfed rather than overfed, which, however, is not necessarily equivalent to the conclusion that they are undernourished, for, broadly speaking, that is seldom the case. Such investigations as have been made to determine whether the poor, as a class, are as liable to cancer, or more so, than the well-to-do, as has abundantly been shown to be the case with tuberculosis, have all been in the negative. In other words, the available evidence is rather to the effect that cancer is chiefly a disease of the well-to-do, and by inference, a disease of civilization, which, broadly speaking, is measured by the attained degree of material wellbeing or widely diffused material prosperity. Exact statistics are, unfortunately, obtainable with difficulty, but a most important contribution to our knowledge has recently been made by the public health department of the city of Edinburgh, which has published the statistics of cancer and tuberculosis according to the rental of the houses occupied by the deceased. It is shown by this inquiry that of the mortality from phthisis, 36.4 per cent of the deaths occurred in houses renting at less than about $50 per annum, 34.6 per cent occurred in houses renting at from $50 to $100, and 17.9 per cent occurred in houses renting at over $100 per annum. The remainder occurred in lodging houses, or the information was not given. In contrast,

of the mortality from cancer only 21.8 per cent occurred in the houses with the lowest rental, 37.0 per cent in the houses with a moderate rental, and 35.9 per cent in the houses with a rental rather indicative of the prosperous and the well-to-do. Furthermore, while 6.9 per cent of the deaths from phthisis occurred in lodging houses, typical of the very poor, only 0.9 per cent of the deaths from cancer occurred in this class of residences. The proportion of deaths with residences not stated was practically the same for both diseases. Restating these rather important conclusions, it would appear that the frequency of cancer deaths was about one-third less in the houses of the poor, but nearly double in the houses of the well-to-do, than in the case of those who died from phthisis, which is generally considered a disease of poverty. These observations suggest the great practical value of a further statistical study of the relation of material wellbeing to cancer and tuberculosis.

### MARITAL CONDITION

Among other important statistical factors which require more extended consideration in the study of the cancer problem is marital condition. A recent investigation by the Registrar-General of England and Wales, extending over a period of three years, seems to warrant the important conclusion that cancer of the breast and ovaries is decidedly more common among single than among married women, when proper correction is made for variations in the age distribution. In contrast, cancer of the uterus, however, is decidedly more common among the married than among the unmarried, but there are reasons for believing that if a further analysis could be made of the precise site of the disease it would be shown that the excess in the mortality of the married is chiefly limited to cancer of the cervix.

### CANCER INCREASE IN OLD AGE

Of all the factors, however, which influence the cancer death rate, age is of the first order of importance. Cancer is essentially a disease of adult life, and of the 80,000 estimated deaths

from cancer in the United States at all ages for the year 1915, approximately 67,600, or 84.5 per cent, occur at ages 45 and over. Since the age distribution of the population varies materially, not only for different countries, states, and cities, but also for different periods of time, it is imperative that, as far as practicable, the age factor should always be taken into account. Unfortunately a recalculation of crude cancer death rates, in conformity to a standard basis of age distribution, is frequently quite difficult and often practically impossible.

The most useful statistics for the purpose of illustrating the influence of age on the cancer death rate, and the changes in the rate, are those for England and Wales, which for the present purpose, however, are limited to the male population and the two years 1901 and 1913. The data are set forth in the table below:

*Mortality from cancer in England and Wales according to age, 1901 con. 1913**

(Rates per 100,000 of population)

| AGE | 1901 | | | 1913 | | | INDEX NUMBER |
|---|---|---|---|---|---|---|---|
| | Population | Deaths | Rates | Population | Deaths | Rates | |
| Under 5..... | 1,855,361 | 66 | 3.6 | 1,952,263 | 74 | 3.8 | 106 |
| 5–14..... | 3,409,963 | 69 | 2.0 | 3,631,918 | 63 | 1.7 | 85 |
| 15–24..... | 3,080,166 | 102 | 3.3 | 3,173,023 | 124 | 3.9 | 118 |
| 25–34..... | 2,485,954 | 236 | 9.5 | 2,900,795 | 365 | 12.6 | 133 |
| 35–44..... | 1,931,944 | 804 | 41.6 | 2,417,420 | 1,084 | 44.8 | 108 |
| 45–54..... | 1,396,210 | 2,040 | 146.1 | 1,753,957 | 3,021 | 172.2 | 118 |
| 55–64..... | 907,945 | 3,353 | 369.3 | 1,120,598 | 5,175 | 461.8 | 125 |
| 65–74..... | 477,868 | 2,958 | 619.0 | 627,744 | 4,934 | 786.0 | 127 |
| 75–84..... | 165,233 | 1,150 | 696.0 | 187,596 | 1,874 | 999.0 | 144 |
| 85 and over... | 17,971 | 113 | 628.8 | 23,690 | 204 | 861.1 | 137 |
| All ages..... | 15,728,615 | 10,891 | 69.2 | 17,789,004 | 16,918 | 95.1 | 137 |

* Males only.

According to this table the cancer death rate for males at all ages increased 37 per cent but the most significant increases in the rate occurred at ages 55 and over, having been, respectively, 25 per cent at ages 55–64, 27 per cent at ages 65–74, 44 per cent at ages 75–84, and 37 per cent at ages 85 and over. The table

warrants the conclusion, which is fully sustained by an analysis of corresponding statistics for the United States and other countries, that the increase in the cancer death rate has occurred largely in the period of advanced adult life. An extended statistical study of the details, by single years, reveals the fact that the increase has been far from uniform at every period of life, but that there have been many important fluctuations and variations suggestive of a real increase rather than an apparent improvement in the rate in consequence of increased accuracy in diagnosis and death certification. To illustrate this point of view more precisely a table is included, giving the rates by divisional periods of life for the male population, by single years, for the entire period 1901–1913. This table is self-explanatory and requires no extended discussion.

*Mortality from cancer in England and Wales according to age, 1901–1913**
(Rates per 100,000 of population)

| AGES | UNDER 35 | 35–44 | 45–54 | 55–64 | 65–74 | 75 AND OVER | ALL AGES |
|---|---|---|---|---|---|---|---|
| 1901..... | 4.4 | 41.6 | 146.1 | 369.3 | 619.0 | 689.4 | 69.1 |
| 1902..... | 4.4 | 39.0 | 146.8 | 359.6 | 635.7 | 707.6 | 69.6 |
| 1903..... | 4.7 | 42.5 | 157.5 | 380.8 | 626.8 | 741.9 | 73.3 |
| 1904..... | 4.7 | 40.1 | 153.1 | 382.6 | 645.6 | 771.2 | 74.3 |
| 1905..... | 5.1 | 39.5 | 150.4 | 383.4 | 664.0 | 801.8 | 75.9 |
| 1906..... | 5.1 | 43.4 | 158.8 | 410.1 | 675.7 | 803.5 | 79.8 |
| 1907..... | 5.2 | 42.4 | 151.9 | 398.4 | 664.8 | 805.1 | 78.6 |
| 1908..... | 5.2 | 41.8 | 161.6 | 401.8 | 700.0 | 845.2 | 82.0 |
| 1909..... | 4.9 | 40.8 | 157.9 | 411.4 | 722.4 | 846.8 | 83.2 |
| 1910..... | 5.4 | 43.6 | 166.9 | 410.1 | 731.0 | 857.8 | 85.7 |
| 1911..... | 5.4 | 41.9 | 171.2 | 426.4 | 763.5 | 897.8 | 89.1 |
| 1912..... | 4.8 | 43.8 | 168.5 | 448.8 | 775.1 | 939.0 | 91.6 |
| 1913..... | 5.4 | 44.8 | 172.2 | 461.8 | 786.0 | 983.5 | 94.7 |

* Males only.

## MORTALITY OF THE WESTERN HEMISPHERE

The present state of international vital statistics is far from being satisfactory and conclusive. Every international comparison is more or less liable to serious errors but particularly so in the case of the countries of practically the entire western

hemisphere. An extended discussion of the local cancer death rate by countries, states, and cities throughout this vast area would obviously be impracticable on the present occasion, but the following observations are presented as a tentative contribution towards a more scientific and extended study of the geographical distribution of cancer throughout the western hemisphere. Combining the available statistics from official sources for the period 1908–1912, the average cancer frequency rates for the principal geographical divisions were as follows:

*Latitude 50° N.–40° N.* includes the principal cities or states of British North America and the northern tier of the registration area of the United States, with a population of approximately 14,400,000. The average cancer death rate for this area was 77.3 per 100,000 of population.

*Latitude 40° N.–30° N.* includes the larger portion of the central and southern tiers of the United States registration area, with a population of approximately 6,000,000. The average cancer death rate for this area was 85.5 per 100,000 of population.

*Latitude 30° N.–10° N.* includes the cities of New Orleans, Havana (Cuba), Mexico City, and Caracas (Venezuela), with a population of approximately 1,275,000. The average cancer death rate for this area was 77.2 per 100,000 of population.

*Latitude 10° N.–10° S.*, entirely within the tropics, includes the cities of Paramaribo (Dutch Guiana), Bogota (U. S. Colombia), and Guayaquil (Ecuador), with a population of approximately 240,000. The average cancer death rate for this area was 82.7 per 100,000 of population.

*Latitude 10° S.–30° S.* includes the principal cities of Brazil, and Santiago del Estero (Argentina), with a population of approximately 1,600,000. The average cancer death rate for this area was 38.2 per 100,000 of population.

*Latitude 30° S.–40° S.* includes the city of Pelotas (Brazil), the two principal cities of the Argentine Republic, and the city of Montevideo (Uruguay), with a population of approximately 2,000,000. The average cancer death rate for this area was 89.8 per 100,000 of population.

## COMPARATIVE RATE OF FREQUENCY

The general conclusion would seem to be justified that there is a decreasing rate of cancer frequency with diminishing distance from the equator. How far this result is attributable to race and primitive conditions of life, rather than to climatological effects, can not be stated at the present time. In a general way, however, it would seem safe to assume that the effect of race is decidedly more pronounced than the result of more obscure climatological conditions such as temperature, rainfall, and humidity. No conclusive investigation has as yet been made to determine the possible correlation of latitude to cancer frequency, but the general evidence available would seem to suggest that latitude is not a pronounced factor in determining the cancer death rate. The following five tables exhibit the cancer death rate for the period 1908–1912, for typical cities of North, Central, and South America.

The first table is for the northern portion of North America and includes nine cities, with an aggregate population estimated for 1912 at 11,507,768, and a range in the cancer death rate from 50.6 per 100,000 of population for the city of Winnipeg, to 105.7 for the city of Boston:

*I. Mortality from cancer in cities of Northern North America, Latitude 50° N.-40° N., 1908–1912*

(Rates per 100,000 of population)

| | LATITUDE | POPULATION (1912) | RATES |
|---|---|---|---|
| Winnipeg | 49° 56′ N. | 159,256 | 50.6 |
| Seattle | 47° 36′ N. | 268,500 | 55.8 |
| Montreal | 45° 30′ N. | 484,400 | 65.4 |
| Toronto | 43° 40′ N. | 414,000 | 72.2 |
| Boston | 42° 22′ N. | 715,711 | 105.7 |
| Chicago | 41° 53′ N. | 2,282,623 | 78.9 |
| New York | 40° 43′ N. | 5,032,821 | 77.1 |
| Pittsburgh | 40° 26′ N. | 550,385 | 66.4 |
| Philadelphia | 40° 00′ N. | 1,600,072 | 85.3 |
| Total | ..... | 11,507,768 | 78.4 |

The second table is for the southern portion of North America and includes ten cities, with an aggregate population estimated for 1912 at 3,179,647, and a range in the cancer death rate from 50.8 per 100,000 of population for the city of Memphis, to 109.7 for the city of San Francisco. In the case of the more southern American cities the race factor is of considerable determining importance:

*II. Mortality from cancer in cities of southern North America, Latitude 40° N.–30° N., 1908–1912*

(Rates per 100,000 of population)

| | LATITUDE | POPULATION (1912) | RATES |
|---|---|---|---|
| Denver | 39° 41′ N. | 229,287 | 83.1 |
| Baltimore | 39° 17′ N. | 568,391 | 89.5 |
| Kansas City | 39° 08′ N. | 265,306 | 79.0 |
| St. Louis | 38° 38′ N. | 709,387 | 81.9 |
| San Francisco | 37° 48′ N. | 431,738 | 109.7 |
| Memphis* | 35° 08′ N. | 136,861 | 50.8 |
| Los Angeles | 34° 05′ N. | 362,541 | 100.9 |
| Charleston* | 32° 46′ N. | 59,437 | 58.8 |
| Savannah* | 32° 05′ N. | 67,228 | 55.9 |
| New Orleans | 30° 00′ N. | 349,471 | 84.9 |
| Total | ..... | 3,179,647 | 87.0 |

* In 1910 the negro population of Memphis was 40.0 per cent of the total; of Charleston, 52.8; of Savannah, 51.1; and of New Orleans, 26.3.

The third table includes the West Indies, Mexico, and Central America, with an aggregate population estimated for 1912 at 1,305,566. To increase the practical value of this table the same has not been limited entirely to cities, but includes a few islands, since in the West Indian island communities the urban and rural populations are considerably merged on account of the fact that the larger cities have, as a rule, the only available facilities for institutional treatment. The range in the cancer death rate of this group is from a minimum of 20.7 per 100,000 of population for the island of Grenada, and 20.9 for the island of St. Lucia, to 102.7 for the city of Havana.

*III. Mortality from cancer in the West Indies, Mexico, and Central America, Latitude 32° N.–10° N., 1908–1912*

(Rates per 100,000 of population)

| | LATITUDE | POPULATION (1912) | RATES |
|---|---|---|---|
| Bermuda | 32° 00′ N. | 19,392 | 54.1 |
| Havana | 23° 09′ N. | 353,509 | 102.7 |
| Mexico City | 19° 26′ N. | 491,500 | 49.5 |
| Danish West Indies | 18° 20′ N. | 26,742 | 91.0 |
| St. Lucia | 14° 01′ N. | 49,205 | 20.9 |
| Windward and Leeward Islands | 14° 00′ N. | 237,041 | 27.0 |
| San Salvador | 13° 44′ N. | 60,000 | 58.0 |
| Grenada | 12° 02′ N. | 68,177 | 20.7 |
| Total | ..... | 1,305,566 | 56.8 |

The fourth table is for the northern portion of South America, the six cities represented having a combined population for the year 1912 of 698,183. The range in the cancer death rate is from a minimum of 21.8 for Bahia, to a maximum of 104.8 for Caracas.

*IV. Mortality from cancer in cities of northern South America, Latitude 10° N.–20° S., 1908–1912*

(Rates per 100,000 of population)

| | LATITUDE | POPULATION (1912) | RATES |
|---|---|---|---|
| Caracas | 10° 31′ N. | 75,000 | 104.8 |
| Paramaribo | 5° 39′ N. | 35,000 | 95.6 |
| Bogota | 4° 35′ N. | 121,257 | 89.7 |
| Guayaquil | 2° 11′ S. | 80,000 | 59.6 |
| Bahia | 13° 00′ S. | 300,000 | 23.2 |
| La Paz | 16° 30′ S. | 86,926 | 21.8 |
| Total | ..... | 698,183 | 52.7 |

The fifth table is for the cities of the southern portion of South America, the seven cities represented having an aggregate population for the year 1912 of 3,257,569. The range in the cancer death rate is from a minimum of 36.5 per 100,000 of population for Bello Horizonte, to a maximum of 116.9 for the city of Montevideo.

*V. Mortality from cancer in cities of southern South America, Latitude 20° S.–40° S., 1908–1912*

(Rates per 100,000 of population)

| | LATITUDE | POPULATION (1912) | RATES |
|---|---|---|---|
| Bello Horizonte | 20° 00′ S. | 39,845 | 36.5 |
| Rio de Janeiro | 22° 54′ S. | 710,600 | 42.5 |
| Sao Paulo | 23° 38′ S. | 400,000 | 45.4 |
| Santiago del Estero | 27° 48′ S. | 20,580 | 37.5 |
| Buenos Aires | 34° 36′ S. | 1,383,663 | 85.5 |
| Montevideo | 34° 54′ S. | 355,017 | 116.9 |
| Santiago de Chile* | 35° 00′ S. | 347,864 | 71.1 |
| Total | ..... | 3,257,569 | 72.5 |

* Population for 1909; rate for period 1905–1909.

The foregoing rates are largely in the nature of an approximation to the exact truth. It, however, may safely be asserted that the margin of error is not of sufficiently serious importance to invalidate general conclusions based upon the data presented, which, without exception, are derived from official sources. It is practically a foregone conclusion, however, that in the case of communities or cities with very low cancer death rates, the chances of error in death certification and completeness of registration are more serious than in the case of communities or cities with high cancer death rates, but the range in the rates is so considerable that there can be no question of doubt but that underlying local causes account for the differences, which are too pronounced to be due in any material degree to errors in clinical diagnosis, death certification, and completeness of registration.

These data are illustrated graphically in Figure 2.

## VARIATIONS BY ORGANS AND PARTS

It is realized that this statement requires some amplification, and in support thereof the following observations on the comparative rate of cancer frequency, with a due regard to sex and organs or parts affected, for a few selected cities of North, Central, and South America are presented:

# International Statistics of Cancer Mortality, 1908–1912

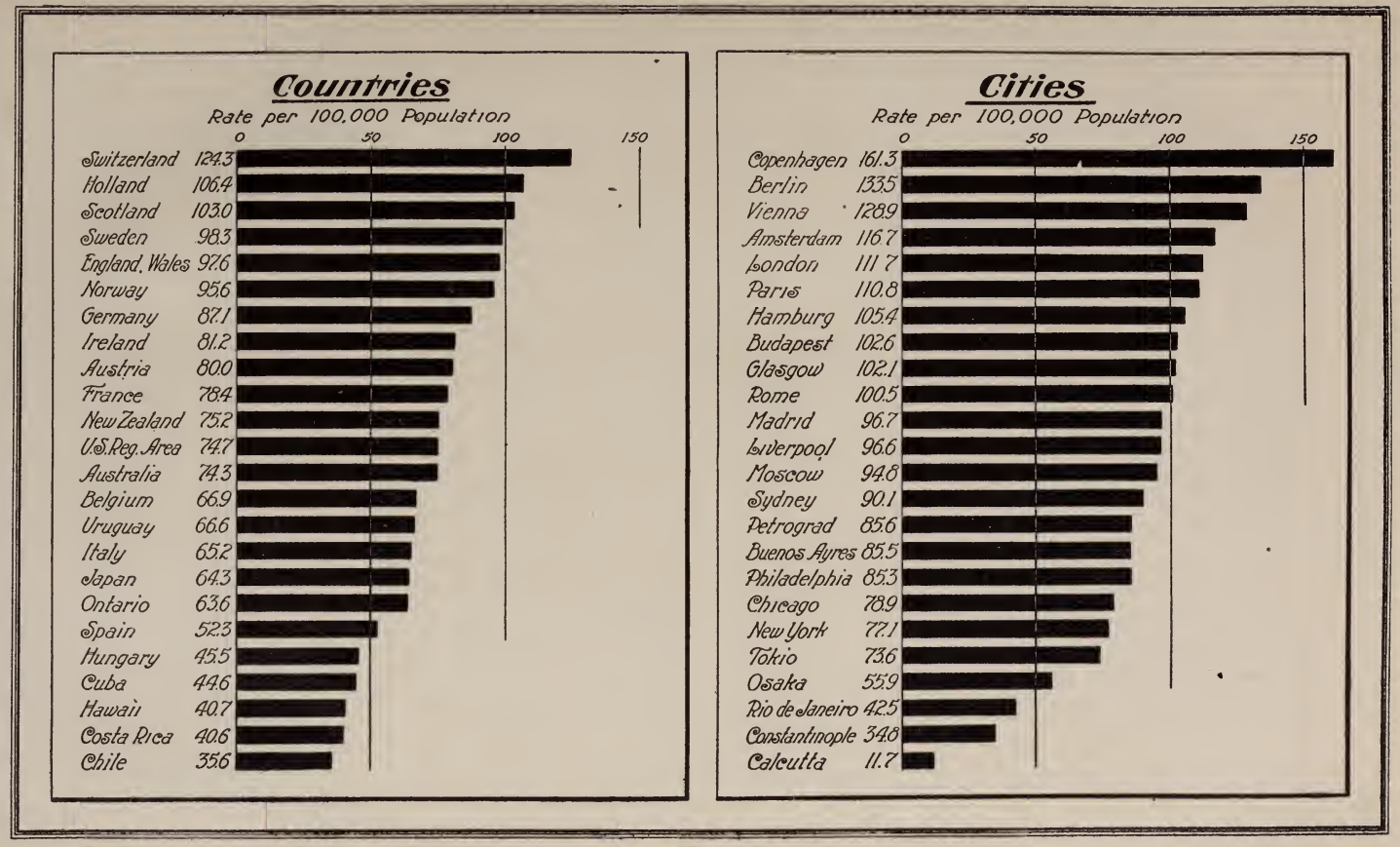


FIGURE 2

*Mortality from cancer in cities of North, Central, and South America, by organs and parts, according to sex*

(Rates per 100,000 of population)

| ORGAN OR PART | NORTH AMERICA | | | CENTRAL AND SOUTH AMERICA | | | |
|---|---|---|---|---|---|---|---|
| | New York | *New Orleans | San Francisco | City of Mexico | Rio de Janeiro | Montevideo | Buenos Aires |
| MALES | | | | | | | |
| Buccal cavity | 4.7 | 7.8 | 10.6 | 2.7 | 3.9 | 5.5 | 6.5 |
| Stomach and liver | 30.9 | 27.5 | 54.1 | 7.3 | 10.0 | 77.9 | 53.5 |
| Peritoneum, intestines and rectum | 10.3 | 6.4 | 13.7 | 2.4 | 1.7 | 7.3 | 4.5 |
| Skin | 1.8 | 1.5 | 2.5 | 1.8 | 2.0 | 1.3 | 2.0 |
| Other or not specified organs | 15.1 | 32.1 | 20.9 | 13.1 | 13.6 | 35.0 | 31.5 |
| All organs and parts | 62.8 | 75.3 | 101.8 | 27.3 | 31.3 | 127.0 | 98.1 |
| FEMALES | | | | | | | |
| Buccal cavity | 0.9 | 2.2 | 1.1 | 1.3 | 1.2 | 1.2 | 1.1 |
| Stomach and liver | 30.5 | 23.2 | 40.1 | 9.4 | 4.1 | 47.3 | 23.4 |
| Peritoneum, intestines and rectum | 12.1 | 6.8 | 17.6 | 4.0 | 1.8 | 8.7 | 4.5 |
| Generative organs | 23.4 | 35.6 | 32.6 | 36.3 | 15.4 | 23.3 | 18.4 |
| Breast | 13.7 | 12.0 | 20.2 | 3.5 | 3.6 | 7.2 | 4.7 |
| Skin | 1.1 | 0.6 | 2.1 | 0.8 | 0.9 | 1.2 | 0.8 |
| Other or not specified organs | 9.8 | 16.9 | 10.6 | 13.3 | 18.8 | 21.1 | 18.2 |
| All organs and parts | 91.5 | 97.3 | 124.3 | 68.6 | 45.7 | 110.0 | 71.2 |

* The data for New Orleans are for the white population only.

The table provides sufficient material for the purpose of illustrating the very wide degree of variation in the local incidence of cancer according to organs or parts of the body affected. It can not well be questioned, for illustration, that the status of medical practice in Buenos Aires is not so very much different from the attained degree of medical and surgical proficiency in the city of New York. The table shows that the general cancer death rate of males in the city of New York was 62.8 per 100,000 of population, against 98.1 in the city of Buenos Aires. It could not well be maintained that the physicians of Buenos Aires diagnose malignant disease with much greater accuracy than the physicians of the city of New York, yet cancer among men is shown to be approximately 50 per cent more frequent in Buenos Aires than in New York City, and upon further analysis it

appears that most of this excess is due to the higher degree or rate of frequency occurrence in the case of cancer of the buccal cavity, stomach and liver, skin, and ill-defined or nonspecified organs or parts. The disease, however, is less frequent in Buenos Aires than in New York City in the case of cancer of the peritoneum, and intestines and rectum. For this group of cancers the death rate for the city of New York is more than twice the male corresponding rate for the city of Buenos Aires.

In marked contrast, the female cancer death rate of the city of New York is 91.5 per 100,000 of population, against a corresponding rate of 71.2 for the city of Buenos Aires. Considered in detail, it appears that the only form of cancer more common among the women of Buenos Aires is cancer of the buccal cavity, but the excess is very slight. The excess in the rate for ill-defined or not specified forms of cancer can not be relied upon as entirely conclusive. In a general way, all important specified forms of cancer are less common among the women of Buenos Aires than among the women of New York City, and particularly so is this the case in cancer of the peritoneum, intestines and rectum, and the female breast. The analysis would seem to prove that the differences in the rates are due to local conditions, chiefly, no doubt, variations in habits of life and the possible effect of race and climate, rather than to pronounced or numerically important errors of clinical diagnosis and death certification.

These data are illustrated in Figure 4.

### CANCER IN THE UNITED STATES AND URUGUAY

As a further contribution to this very interesting aspect of the geographical study of cancer frequency, and as evidence of the great practical value of an extended statistical analysis of the official returns, the following comparative table is included for the United States registration area and for Uruguay:

*Mortality from cancer in the United States registration area and the Republic of Uruguay, by organs and parts, 1906–1910*

(Rates per 100,000 of population)

| | MALES | | FEMALES | | MALES AND FEMALES | |
|---|---|---|---|---|---|---|
| | United States | Uruguay | United States | Uruguay | United States | Uruguay |
| Buccal cavity | 4.2 | 3.6 | 0.9 | 0.4 | 2.6 | 2.0 |
| Stomach and liver | 27.6 | 44.4 | 29.0 | 26.5 | 28.3 | 35.6 |
| Peritoneum, intestines and rectum | 7.1 | 4.2 | 10.5 | 4.9 | 8.8 | 4.6 |
| Female generative organs | | | 22.1 | 12.2 | 10.8 | 6.0 |
| Female Breast | | | 13.3 | 3.7 | 6.5 | 1.8 |
| Skin | 3.4 | 1.6 | 1.9 | 0.5 | 2.7 | 1.1 |
| Other or not specified organs | 13.4 | 19.4 | 12.4 | 10.2 | 12.9 | 14.9 |
| All organs and parts | 55.7 | 73.2 | 90.1 | 58.4 | 72.6 | 66.0 |

The general cancer death rates of the two countries are not very far from being about the same. The rate for Uruguay was 66.0 per 100,000 of total population during the period under consideration, against a rate of 72.6 for the United States registration area. Considered, however, by four groups of specified organs or parts, it appears that the rate for cancer of the stomach and liver was 28.3 per 100,000 of total population for the United States registration area, against 35.6 for Uruguay. It could not well be maintained, without the risk of successful contradiction, that cancer of the stomach and liver is less accurately diagnosed in the United States than in the South American republic. The mortality from cancer of the skin was 2.7 per 100,000 of total population in the United States registration area, against a rate of 1.1 for Uruguay. Since this form of cancer is the most easily diagnosed, it is reasonable to suppose that the differences in the rate can not be attributed to the serious shortcomings of medical practice in the South American republic.

These data are illustrated in Figures 1 and 3.

### FEMALE GENERATIVE ORGANS AND BREAST

The mortality from cancer of the female generative organs was 22.1 per 100,000 of female population for the United States registration area, against a rate of 12.2 for Uruguay; and the

# Cancer Mortality — Uruguay

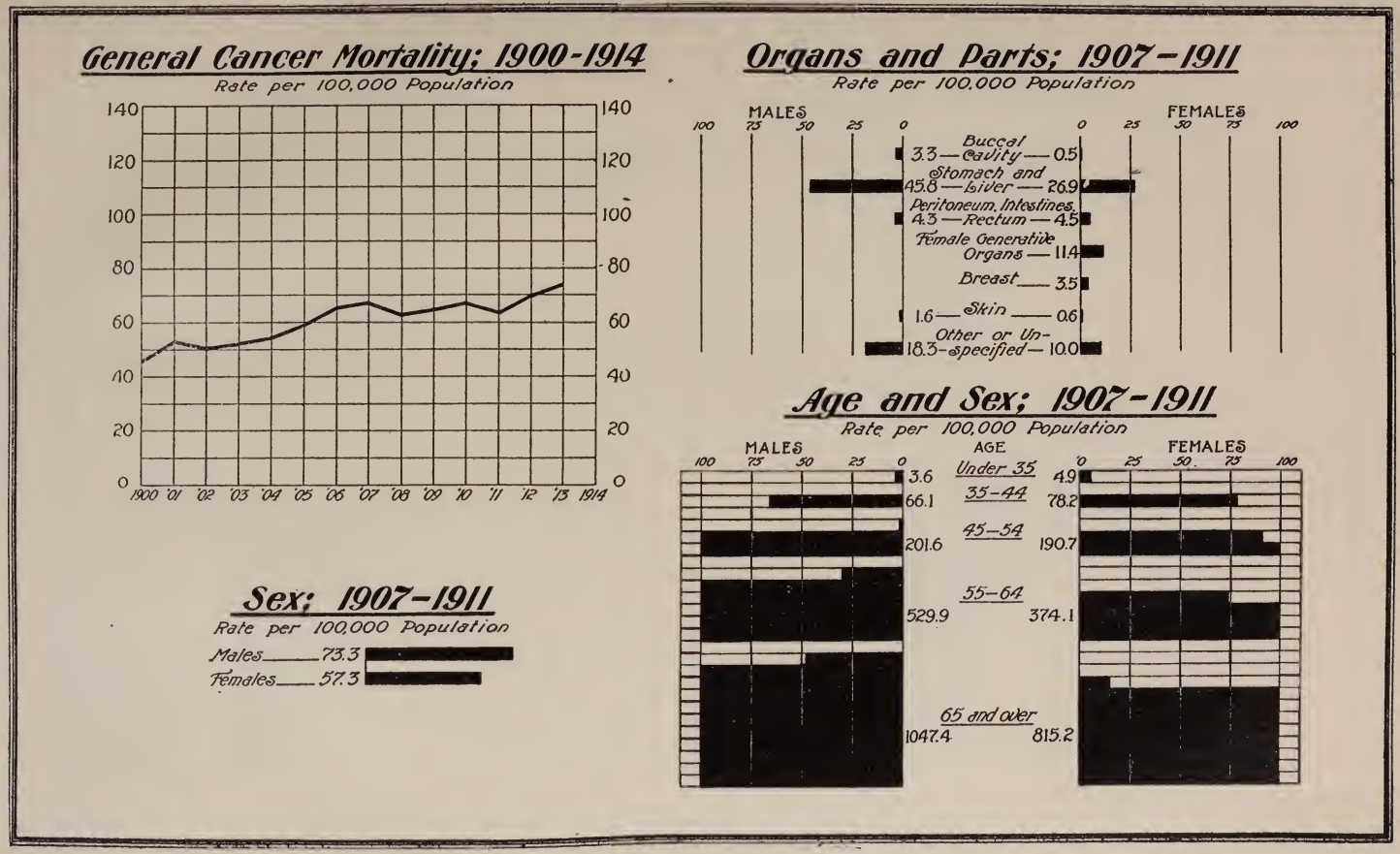


FIGURE 3

corresponding rates for cancer of the female breast were 13.3 for the United States registration area, against only 3.7 for Uruguay. No evidence is available to prove that cancer of the female generative organs is less accurately diagnosed in Uruguay than in the United States, if the higher rate for cancer of the stomach and liver in Uruguay can be relied upon as evidence that the diagnosis of a somewhat similar group of generally inaccessible cancers is as accurately made as in the United States.

The very low mortality from cancer of the female breast in Uruguay can not be accepted as proof of inaccuracy or incompleteness of diagnosis, since this is one of the most easily diagnosed forms of malignant disease. By way of further illustration, it may be stated in this connection that the mortality from cancer of the female breast is 4.5 per 100,000 of female population for the island of Cuba, but only 1.8 for Japan. In contrast, the rate attains to extremely high proportions in England and Wales, where it is 17.9; and in Scotland, where it is 15.4. Since the corresponding rate for the United States registration area is only 13.3, it would follow that if the low rate for Uruguay is to be considered evidence of inaccuracy or incompleteness of diagnosis, then the same conclusion applies to the United States and Japan, and a number of other countries for which, as far as known, the registration returns are as trustworthy as for England and Wales, Scotland, Switzerland, and the Australian commonwealth. In fact, it may be said further in this connection, that the mortality from cancer of the breast for Bavaria, where the general accuracy of diagnosis can not be questioned, was only 9.1 per 100,000 of female population, and for Holland only 9.6, against, as said before, corresponding rates of 17.9 for England and Wales, and 15.4 for Scotland.

These data are illustrated in Figure 4.

## ACCURACY OF DIAGNOSIS

The foregoing observations are called for in defense of the practical use of the general cancer mortality statistics of the western hemisphere as an approximate indication of the geographical distribution of the disease throughout this vast area,

# Cancer Mortality by Organs and Parts, 1908—1912

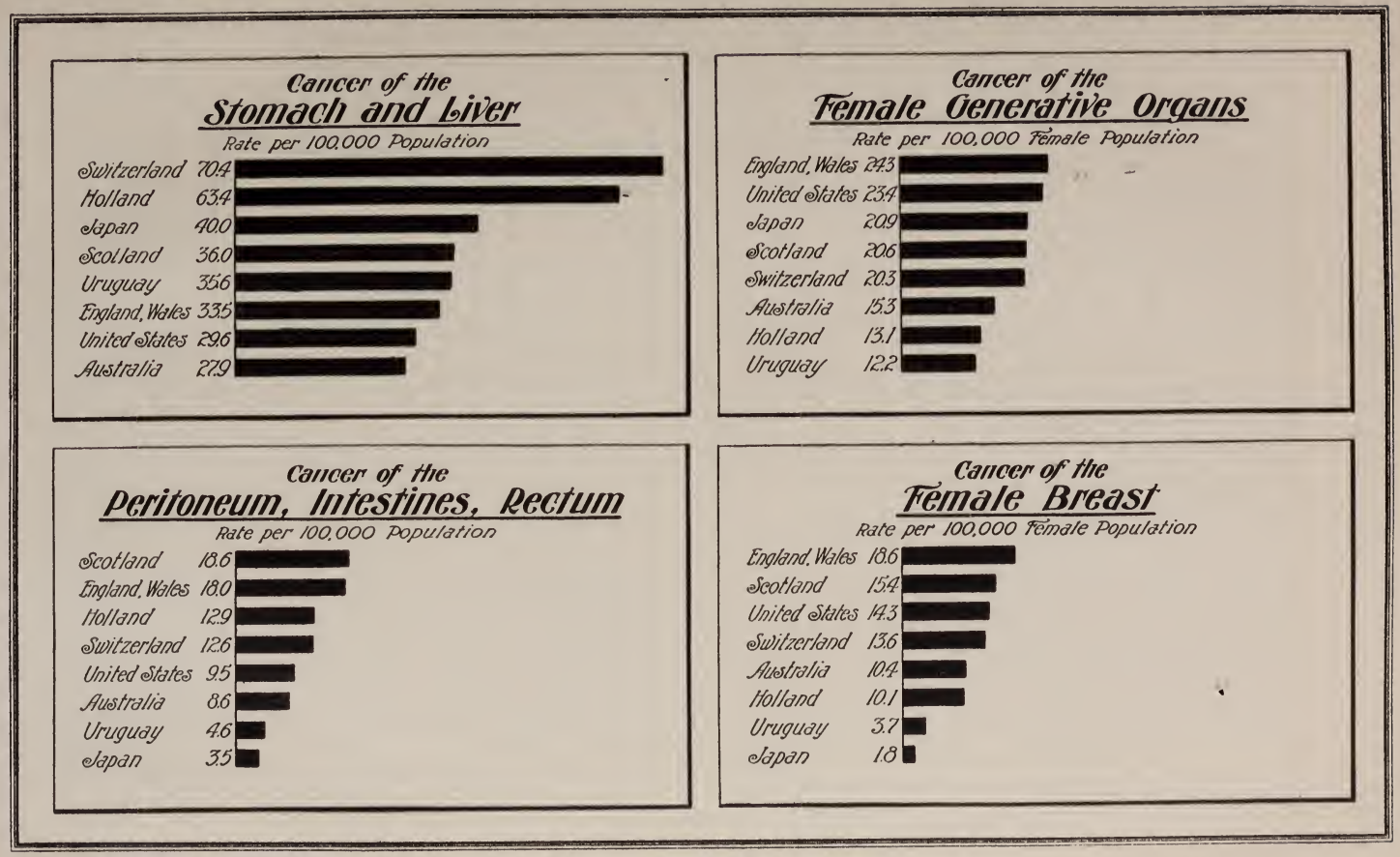


FIGURE 4

and the modification of local cancer death rates by population, climatological conditions, habits of life, etc., as the case may be. Those who are responsible for the charge that methods of death certification are grossly defective, and that in a large number of cases the clinical diagnosis is inaccurate or seriously at fault, rely upon fragmentary data and not upon the required statistical evidence in conformity to the first and fundamental law of all statistical inquiries, and that is the law of large numbers. Most of the contributions to the medical literature on accuracy in death certification bear intrinsic evidence of superficial consideration and indifference to accepted principles of statistical inquiry. It is as wrongful a procedure on the part of a physician not trained in statistical methods to bring forward statistical arguments and far reaching assertions based upon mere figures or data not conforming to the requirements of statistical science, as for a statistician superficially informed concerning medical matters to pass judgment on involved problems of pathology or therapeutics. It is unquestionably of the first order of importance that the clinical diagnosis and methods of death certification should be further improved, and no one with any knowledge of the facts but will admit that present methods throughout the world are far from perfect or ideal; but the strongest possible objections lie against the increasing practice on the part of superficial and ill-informed medical writers, to indict on the one hand the entire practice of medicine and surgery as being little short of quackery, and on the other, the statistical method in medicine as a delusion and a fraud. The cause of neither medicine nor statistics is advanced by such amateurish contributions, but a vast amount of harm is done to both medicine and statistics by their publication in scientific periodicals, official health reports, or in the medical press.

### URGENCY OF QUALIFIED STATISTICAL RESEARCH

The foregoing observations are chiefly intended to emphasize the urgency and practical utility of further statistical research into the geographical incidence of cancer throughout the western hemisphere. Conceding the rather doubtful accuracy and com-

pleteness of the returns for certain countries and islands largely inhabited by native races, it nevertheless seems reasonable to maintain that if malignant disease were actually as common in these areas as in the more civilized portions of the globe, the recorded rate of frequency would be much higher than is actually the case. Recalling the wide disparity in the comparative rate of incidence, by organs and parts, met with in such cities as New York and San Francisco, or Chicago and New Orleans, it would seem utterly incredible that these differences should be the result of serious errors in diagnosis or inaccuracies in death certification, instead of, as is more probable, due to pronounced variations in the existing conditions of life, chiefly habits and diet, which in part at least are the equivalent of material well being and poverty. While cancer occurs among animals under domestication, or under exceptional laboratory conditions, it is well known that malignant disease is relatively infrequent among wild animals in captivity. Conversely, there are no reasons why native races should be peculiarly or exceptionally liable to malignant disease, and, as a matter of fact, the most careful medical observers, living for many years among primitive peoples, have invariably reported cancer to be of comparatively rare occurrence among them. There are, therefore, convincing reasons for believing that a thoroughly specialized statistical cancer research into the precise geographical incidence of malignant disease throughout the western hemisphere would prove of much practical value and possibly of far-reaching importance to the cause of cancer control.

In contrast to the comparative rarity of cancer in many of the countries and islands of Central and South America, the discussion draws attention to the excessive frequency of the disease in such cities as Buenos Aires and Montevideo, where it has been shown that cancer is even more common than in cities of corresponding size in the northern portion of the western hemisphere. In this direction also the outlook is encouraging that further statistical research, amplified by medical and anthropological studies, and most of all by thoroughly qualified studies of metabolism, diet, and habits of life, would yield results of

considerable practical importance. Accepting as conclusive the recorded rate of excessive cancer frequency for many of the countries and states of the Pan-American Union, it would furthermore seem of the utmost urgency that the attention of these countries should be directed to the principles and methods of the American Society for the Control of Cancer, as a first step in the direction of an effective public education in the essential cancer facts and a prerequisite for an ultimate reduction in the mortality from malignant disease throughout the entire western hemisphere.

# THE EFFECT OF PHLORIDZIN ON TUMORS IN ANIMALS[1]

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*From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director*

Received for publication, December 3, 1915

Following the report of Benedict and Lewis,[2] in 1914, of the cure of malignant tumors in rats by the induction of diabetes with phloridzin, the experiments here described were undertaken for the purpose of ascertaining to what extent the results of these investigators could be duplicated in large series of animals bearing the tumor with which they had worked, as well as of animals bearing other types of neoplasms.

Benedict and Lewis used 40 animals inoculated with the Buffalo rat sarcoma. These animals were placed on a carbohydrate-free diet and injected every second or third day with 0.2 gm. of phloridzin in olive oil. The authors observed "a rapid and complete disappearance of all tumors which at the beginning of treatment did not exceed 20 x 25 mm. in size." They failed to state how long after inoculation the treatment was begun, and to mention the age of the animals; and they gave no charts to show whether or not those tumors which disappeared under treatment had already begun to decrease in size when the injections of phloridzin were started. On the basis of their experiments a case of sarcoma in man was treated with phloridzin with an unfavorable result.

The tumors used in the experiments to be described were: the Buffalo rat sarcoma, Crocker Fund sarcoma No. 180, and seven spontaneous mammary carcinomata of mice.

[1] A preliminary report of the experiments herein described was presented to the Society of Experimental Biology and Medicine, March, 1915.

[2] Benedict and Lewis: Proc. Soc. Exper. Biol. and Med., 1914, xi, 134.

The Buffalo rat sarcoma gives about 90 per cent of positive inoculations, grows fairly rapidly, though the rate is subject to variation, and in many cases develops tumors more than half the size of the host. It has a marked tendency to spontaneous absorption, metastasizes seldom, and rarely ulcerates, but shows great variations in both growth and absorption. In different boxes containing the same series and strain, under apparently the same conditions, the rats in one will have large, rapidly growing tumors, while those in another will bear only small, slowly growing, or receding tumors. In the laboratory record of stock tumors of the Buffalo rat sarcoma extending over fifteen months there were 450 tumor-bearing animals, 40 per cent of which showed partial or complete absorption of the tumor before the death of the animal. The percentage of tumors undergoing absorption varies greatly from time to time. Sometimes nearly every member of a series shows spontaneous regression, so that considerable care has to be exercised to avoid losing the laboratory stock. Absorption, if it occur, usually begins about the time of the third routine charting, i.e., about twenty-four days after inoculation, and is generally progressive. As a routine at the Crocker Fund, tumors are charted in silhouette ten days after inoculation and subsequently every week.

The Crocker Fund mouse sarcoma No. 180 is a richly cellular growth. It gives over 95 per cent of successful inoculations, grows rapidly, and invades the muscles, ulcerating early, as a rule. In cases in which it does not ulcerate it may exceed the host in size. Metastases are frequent. No case of absorption has been observed. The tumors are often the seat of dry gangrene or sometimes undergo such extensive ulceration that only a narrow margin of tumor tissue persists. The resulting contraction of the cicatrix gives the appearance, in silhouette, of absorption, but the remaining tumor tissue continues to grow and invade, the growth regaining its former size if the animal does not die from the ulceration.

In these experiments the animals were injected, usually in the left axilla, with phloridzin, in suspension in olive oil. Treatment was begun, as a rule, at the time of the second charting,

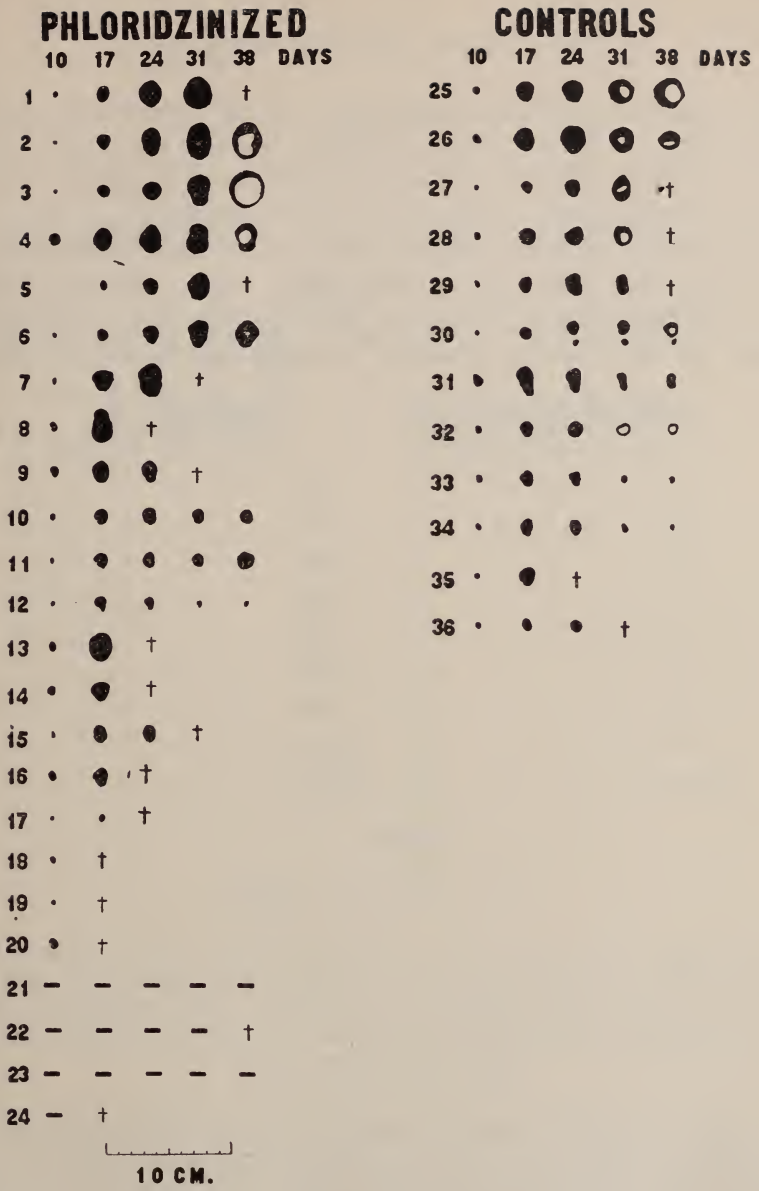


FIG. 1. Experiment $\frac{\text{BRS}}{15\ \text{A}}$

On January 7, 1915, 36 rats were inoculated in right axilla with 0.02 gm. by needle method. On January 24, rats 1 to 24 were given injections of 0.0025 to 0.003 gm. phloridzin in left axilla. Controls: rats 25 to 36. The average size of tumors at any given charting is greater among the controls. Only one tumor in treated animals shows decrease in size as compared to three among the controls.

or seventeen days after inoculation. A few cases were put on treatment ten days after inoculation, while a few others were first rendered diabetic and then inoculated. Animals of from seven to ten weeks of age were used throughout.

The rats were given 0.0025 to 0.003 gm. of the phloridzin in oil and the mice 0.001 gm. of the same, every second or third day. Larger doses, up to 0.2 gm. for the rats, and proportionately larger for the mice, as given by Benedict and Lewis, were tried,

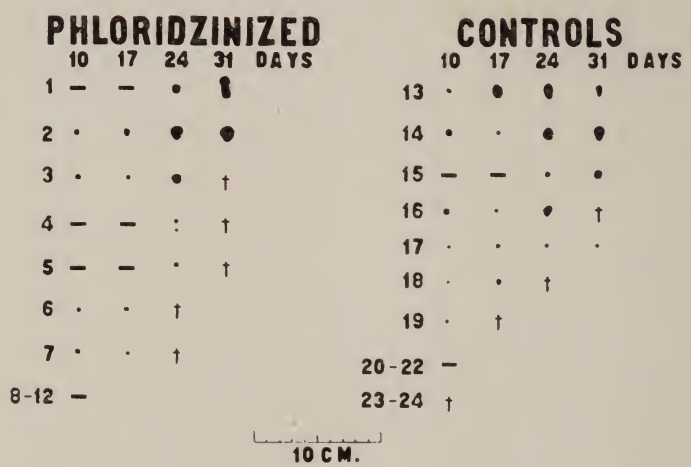


FIG. 2. Experiment $\frac{\text{BRS}}{15\text{ B}}$

On January 8, 1915, 24 rats were inoculated in right axilla with 0.02 gm. by needle method. On January 25, rats 1 to 12 were given injections of 0.003 gm. phloridzin in left axilla; repeated every third day. Small percentage of positive inoculations; very few animals survived. No noticeable difference can be made out between the treated and the control series.

but the animals died in such numbers before a sufficient time had elapsed to observe the effect of the treatment on the tumors, that a dose more compatible with the life of the host, which would yet prove efficient in producing diabetes, had to be used.

All treated animals were kept rigidly on a diet of meat and lard, while the control animals were given the regular laboratory diet of dry bread and vegetables. From time to time, at the end of the second or third day period following injections of the

phloridzin, the collected urines were examined for sugar with Fehling's solution and were found to give a positive reaction in the case of the treated animals on the carbohydrate-free diet,

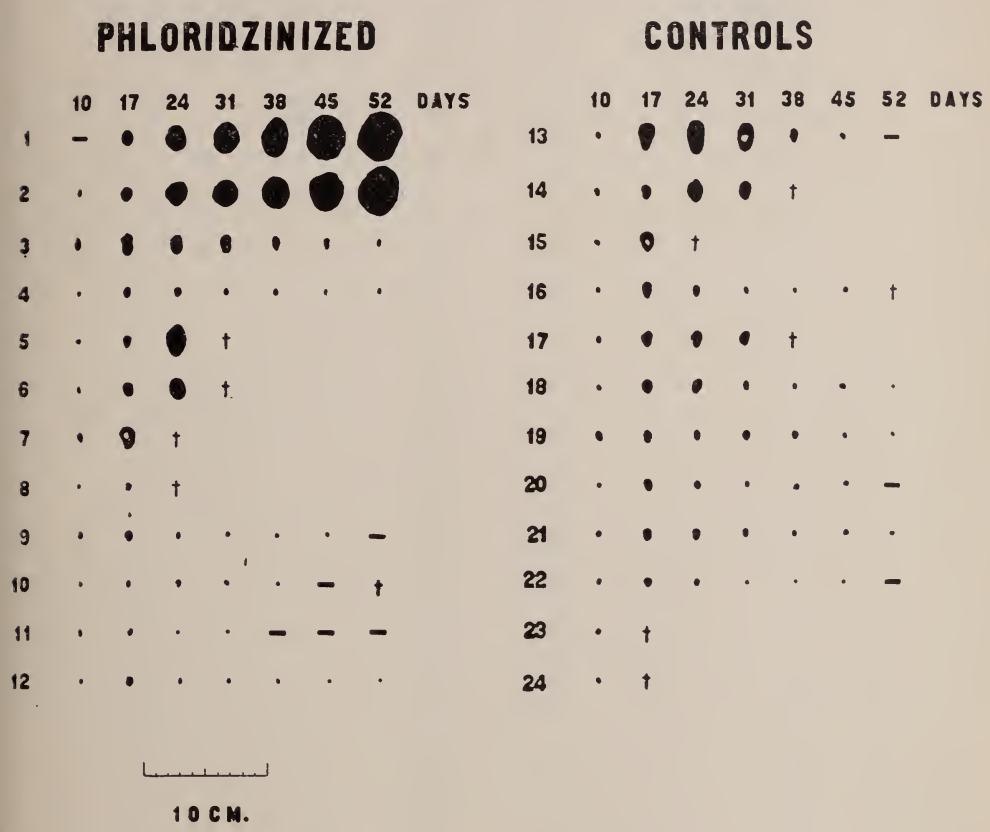


FIG. 3. Experiment $\frac{\text{BRS}}{15\ \text{C}}$

On January 9, 1915, 24 rats were inoculated in right axilla with 0.02 gm. by needle method. On January 26, rats 1 to 12 were started on injections of 0.0025 to 0.003 gm. phloridzin in left axilla. Percentage of absorption is great in both groups but greater in controls. The large size of some of the tumors among the treated animals of this group is striking as compared with the controls.

while the urine of the untreated animals as well as a phloridzin solution gave a negative reaction. The animals under treatment rapidly became emaciated, the fur roughened, and they

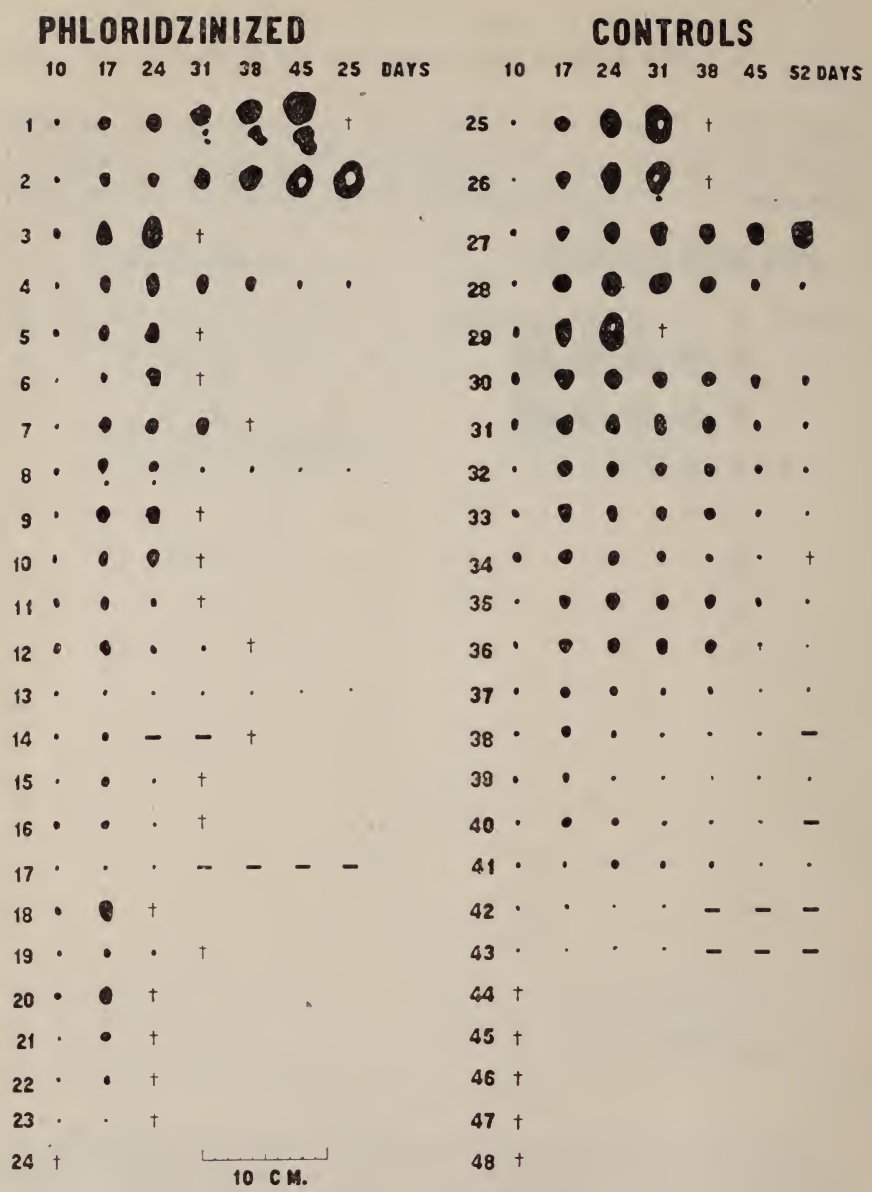


FIG. 4. Experiment $\frac{\text{BRS}}{15\,\text{E}}$

On January 14, 1915, 48 rats were inoculated in right axilla with 0.02 gm. by needle method. On February 1, rats 1 to 24 were injected in left axilla with 0.003 gm. phloridzin; repeated every two or three days. The majority of the treated rats were alive seven days after injection, but only seven survived until the second charting after beginning treatment. The large percentage of tumors undergoing absorption among the control animals compared with the treated animals is marked, 15 out of a possible 19.

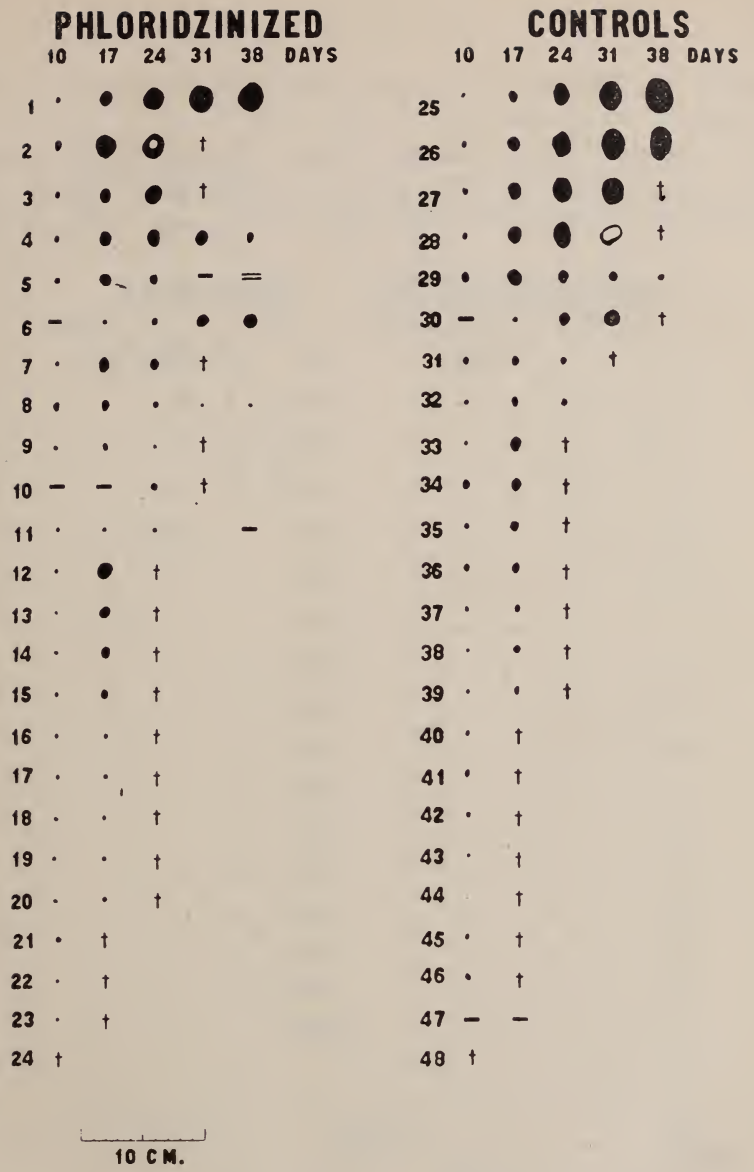


FIG. 5. Experiment $\frac{\text{BRS}}{15\,\text{G}}$

On January 16, 1915, 48 rats were inoculated in right axilla with 0.02 gm. by needle method. On February 3, rats 1 to 24 were started on injections of 0.003 gm. phloridzin in left axilla. Controls: rats 25 to 48. High mortality among all animals, greater among the controls. Tumors slightly larger among the controls.

appeared to be very ill; a great many died soon after the beginning of the treatment.

For the experiments with the Buffalo rat sarcoma, 324 animals were inoculated, with 90.4 per cent of "takes." These were divided into eight series of 24 to 48 animals each. The animals

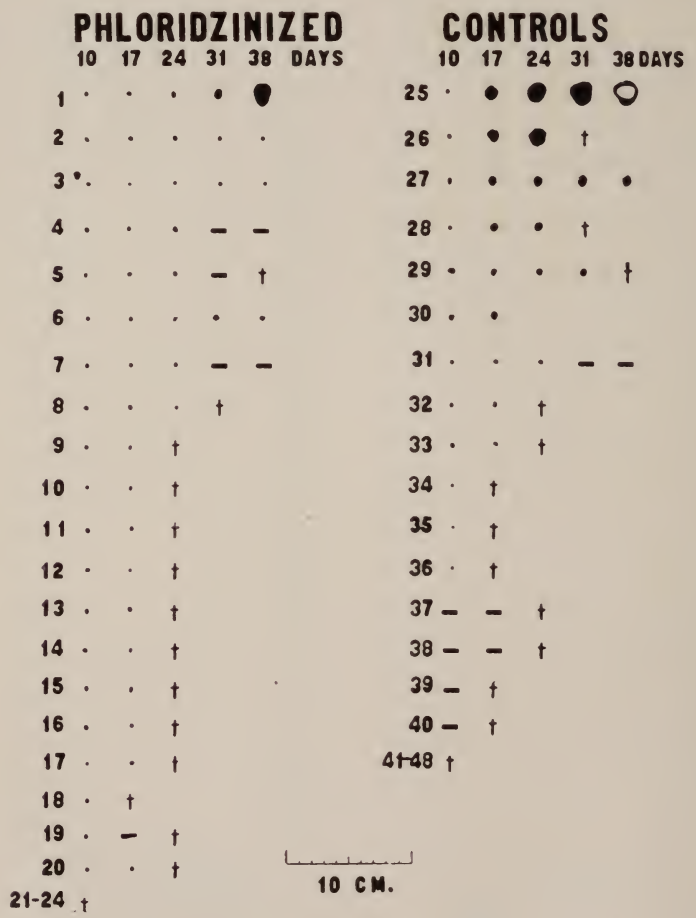


FIG. 6. Experiment $\frac{\text{BRS}}{15\ \text{H}}$

On January 18, 1915, 48 rats were inoculated in right axilla with 0.02 gm. by needle method. On February 4, rats 1 to 24 were started on injections of 0.003 gm. phloridzin in left axilla. Controls: rats 25 to 48. All the animals were in poor condition. Of the few living animals the controls show larger tumors.

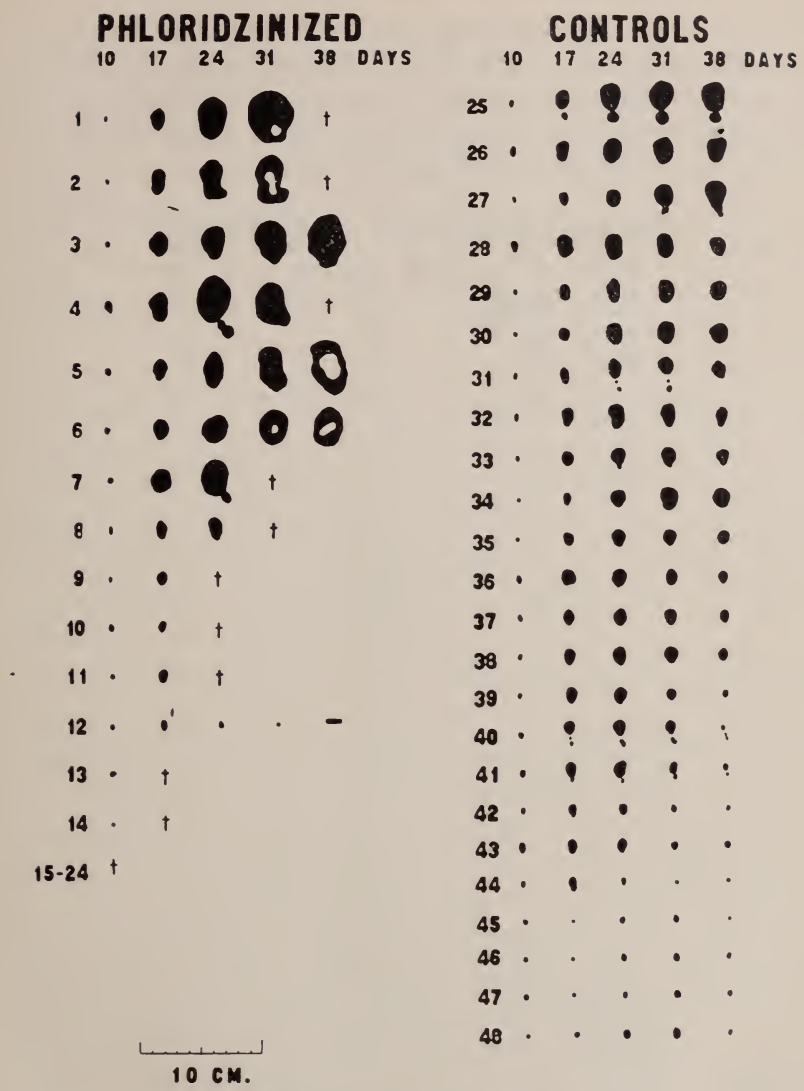


FIG. 7. Experiment $\frac{\text{BRS}}{151}$ 48 rats

On January 19, 1915, rats 1 to 24 were injected in left axilla with 0.0025 gm. phloridzin. On January 20, the entire series 1 to 48 was inoculated with 0.02 gm. in right axilla by needle method. Rats 1 to 25 were given injections every two or three days. Controls: rats 25 to 48. The largest growth of the whole series was obtained among the treated animals of this group. Only one treated tumor shows absorption while all the remaining tumors in animals living beyond two chartings show rapid and extensive growth.

alive one week after the beginning of treatment numbered 170, consisting of 81 treated and 89 controls. Of the 81 treated rats, 30 (37 per cent) showed partial or complete absorption of their tumors before the death of the host, while of the 89 control rats, 52 (58.4 per cent) showed similar absorption.

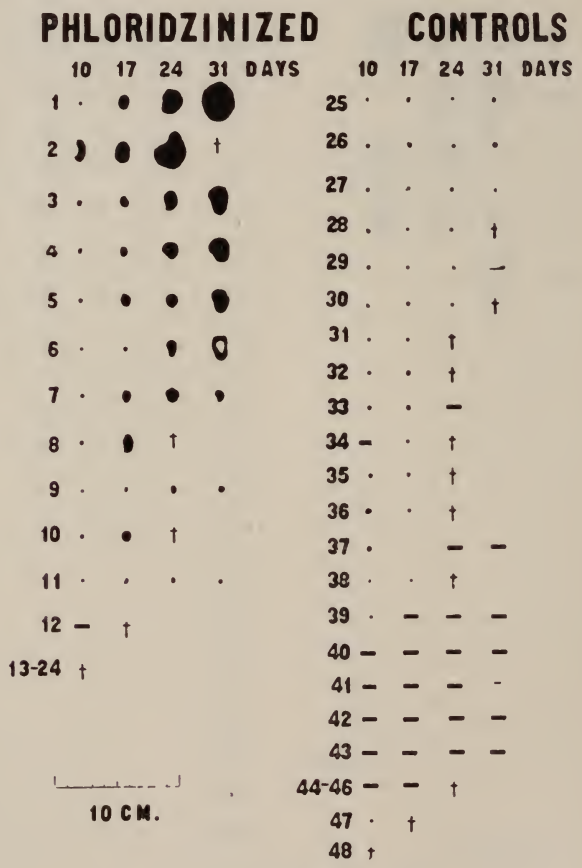


FIG. 8. Experiment $\frac{\text{BRS}}{15\,\text{J}}$

On January 15, 1915, rats 1 to 24 were injected in left axilla with 0.0025 to 0.003 gm. phloridzin; repeated every two days. On February 1, these were inoculated together with 24 normal rats in left axilla with 0.02 gm. tumor by needle method. Of the treated animals only 12 lived until the first charting; of these all but one show positive inoculations with fair or good growth. Among the control animals there are several failures and all the tumors are small.

Figures 1 to 8 show graphically the results obtained, the charts of the treated animals being compared with those of the controls. All the animals inoculated for each experiment are represented in the charts, but only those alive at the first charting (seven days) after beginning treatment, are counted in figuring the results.

The experiments charted were carried out as described above, with the exception of $\frac{BRS}{15I}$ (fig. 7) and $\frac{BRS}{15J}$ (fig. 8). In experiment $\frac{BRS}{15I}$ 24 rats were injected with 0.003 g. phloridzin subcutaneously in the left axilla, and on the following day were inoculated in the right axilla with 0.02 g. of tumor tissue. The largest growth in the whole series of experiments was obtained among the treated members of this group. Only one treated animal showed absorption of its tumor, while the tumors in all the remaining treated animals which lived after the second charting showed rapid and extensive growth.

In experiment $\frac{BRS}{15J}$, 24 rats were treated with phloridzin at regular intervals for ten days, and were then inoculated at the site of the phloridzin injections with 0.02 gm. of tumor tissue. At the same time, 24 normal animals of the same strain were inoculated for controls. This group showed the greatest difference between treated and control animals in rate of growth and percentage of successful inoculation. Eleven of the 12 treated animals which lived until the first charting, ten days after inoculation, showed positive inoculation with good growth, while the control animals showed very small growth of the tumor and there were several failures in inoculation.

The uniformly greater growth, and the smaller percentage among those treated of tumors showing a decrease in size, would suggest a stimulating rather than a curative effect from the phloridzin injections.

The largest of the Buffalo rat sarcomata to undergo absorption was found among the control tumors of experiment $\frac{BRS}{15C}$

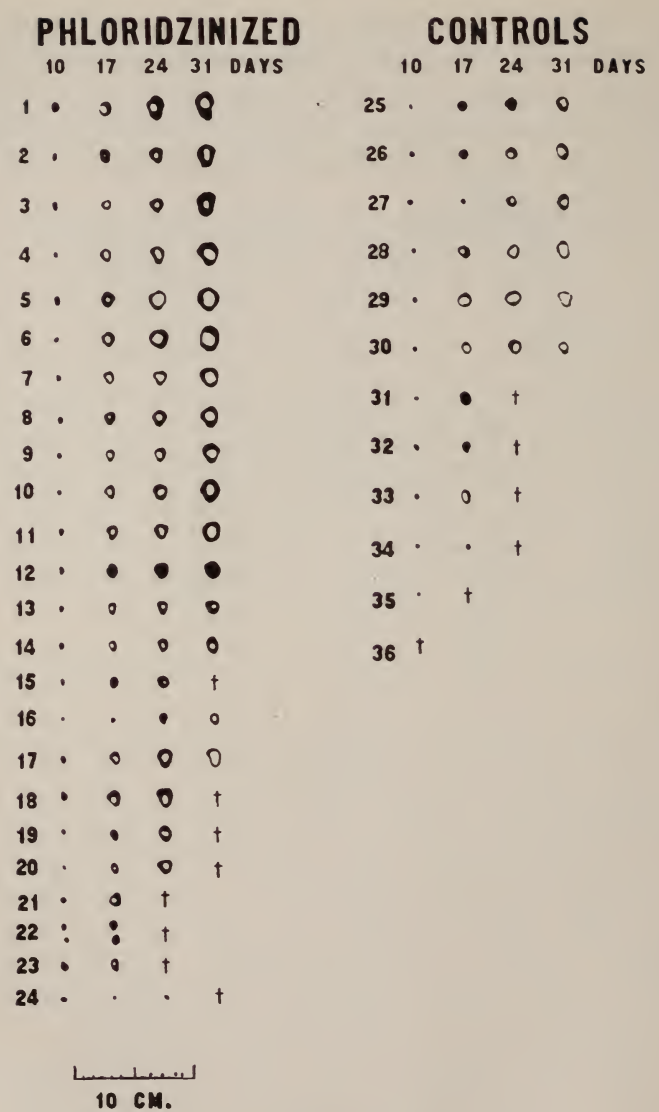


FIG. 9. Experiment $\frac{180}{5\,C}$

On January 7, 1915, 36 rats were inoculated in right axilla with 0.01 by needle method. On January 25, rats 1 to 24 were started on injections of 0.001 gm. phloridzin in left axilla. Controls: rats 25 to 36. Treated animals show markedly larger and more rapidly growing tumors.

(fig. 3); it measured 26 x 14 mm. This is a little smaller than the largest tumor reported as cured by Benedict and Lewis, which measured 20 x 25 mm.

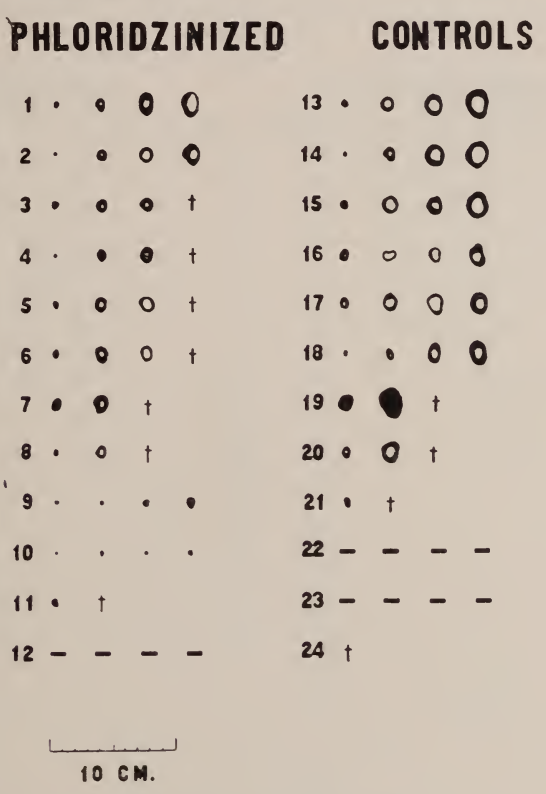


Fig. 10. Experiment $\frac{180}{5\,D}$

On January 8, 1915, 24 mice were inoculated in right axilla with 0.01 gm. by needle method. On January 26, mice 1 to 12 were started on injections of 0.001 gm. phloridzin in left axilla. Controls: mice 13 to 24. Controls show greater growth but average size of tumor in controls is greater than the treated series at time of beginning treatment, i.e., after second charting.

For the experiments with mouse sarcoma No. 180, 396 mice were inoculated, with 97.7 per cent positive. These were divided into ten series of 24 to 48 animals each. Of these, there were alive one week after beginning treatment, 220 animals,

consisting of 97 treated and 123 controls. Of this whole number, 2 of the treated and 2 of the control mice showed decrease in the size of the tumors. These were extensively ulcerated and the decrease in size may be attributed to the contraction of the cicatrix.

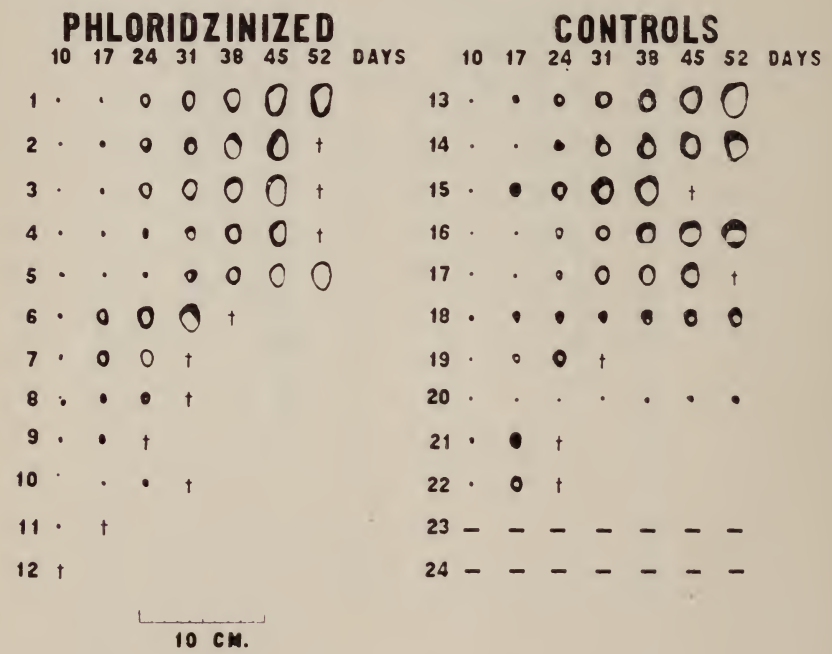


FIG. 11. Experiment $\frac{180}{5G}$

On January 9, 1915, 24 mice were inoculated in right axilla with 0.01 gm. by needle method. January 26, mice 1 to 12 were started on injections of 0.001 gm. phloridzin in left axilla. Controls: mice 13–24. There is a large number of animals of both groups showing extensive growth; no appreciable difference in the two groups.

Figures 9 to 15 give graphically the results obtained with tumor No. 180, the treated being compared with the control animals. Experiments $\frac{180}{5P}$, $\frac{180}{5S}$, and $\frac{180}{5T}$ are not reproduced because of the very great number of deaths among the treated animals.

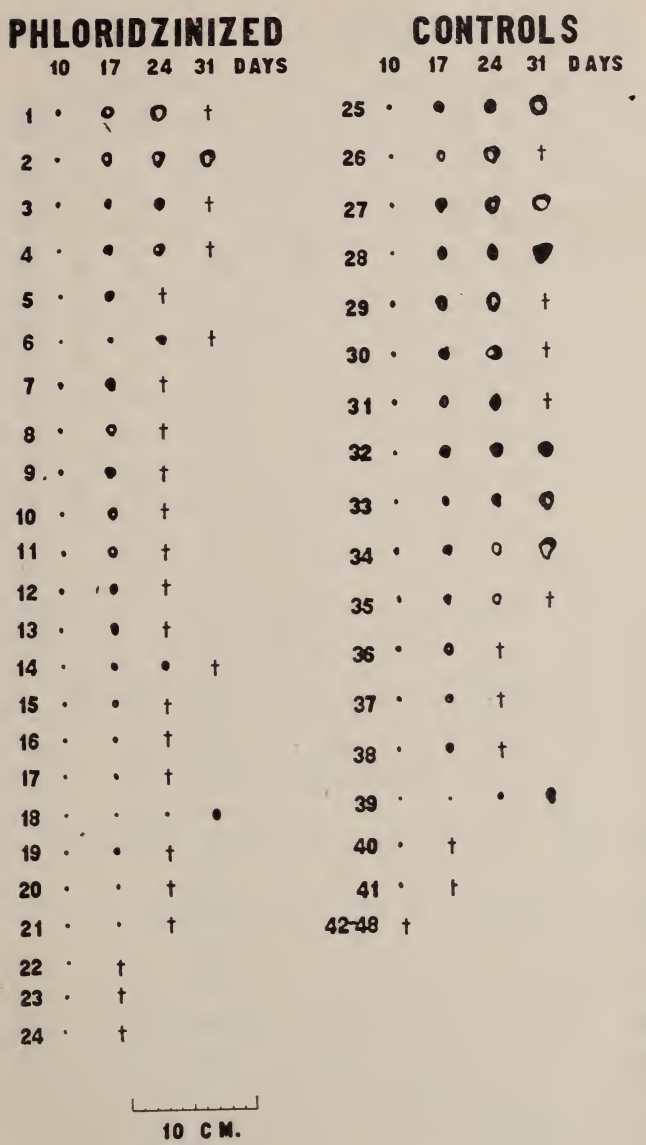


FIG. 12. Experiment $\frac{180}{5\,I}$

On February 1, 1915, 48 mice were inoculated in right axilla with 0.01 gm. by needle method. On January 28, mice 1 to 24 were started on injections of 0.001 gm. phloridzin in left axilla. The mortality among the treated animals is so great that the very few remaining tumors are not a fair comparison for either group.

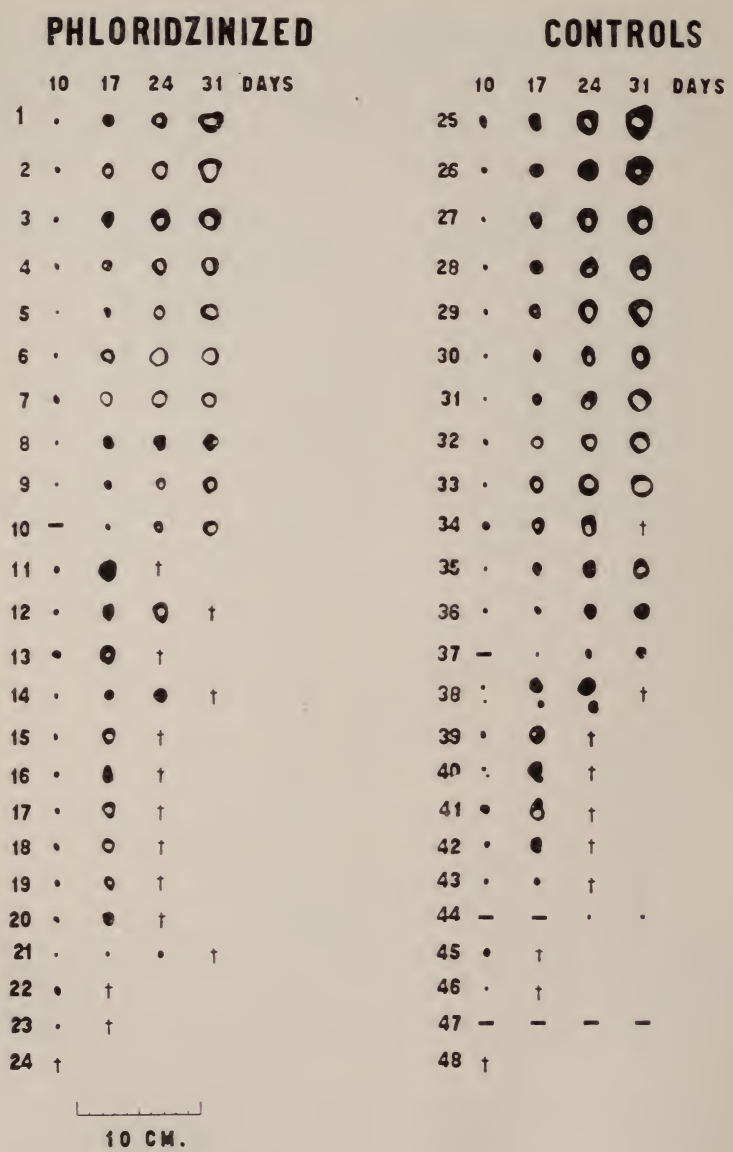


FIG. 13. Experiment $\frac{180}{50}$

On January 15, 1915, 48 mice were inoculated in right axilla with 0.01 gm. by needle method. On February 1, mice 1 to 24 were started on injections of 0.001 gm. phloridzin in left axilla. Controls: mice 25 to 48. The control tumors are appreciably larger than the treated.

In experiment $\frac{180}{5P}$, 24 mice were inoculated and 17 lived until the first charting, when 8 were put on phloridzin injections. Of the 8 animals, only 3 lived one week after beginning treatment, and none until the second week. There were no cases of absorption, or decrease in the size of the tumor.

In experiment $\frac{180}{5S}$, 24 mice were injected with 0.001 gm. phloridzin in the left axilla, and on the following day were inoculated in the right axilla with 0.01 gm. of tumor tissue, together with 24 mice to serve as controls. Of the treated mice, 18 died before the first charting. The remaining mice were all successfully inoculated, but all except one died before the second charting without showing any effect of the treatment on the tumor. Of the 24 control mice, 21 lived for six weeks.

For experiment $\frac{180}{5T}$, 48 mice were inoculated with tumor tissue, with 100 per cent successful inoculations. At the end of ten days, 24 were put on phloridzin injections. Of these, 15 died before the following week, or second charting, and 5 more before the third charting. Of the remaining 4, none showed decrease in size or retardation of growth of the tumor as compared with the tumors of the control animals.

Of the experiments graphically represented, $\frac{180}{5C}$ (fig. 9) and $\frac{180}{5G}$ (fig. 11) showed growth in the treated tumors that equaled or exceeded that in the controls. In experiment $\frac{180}{5D}$ (fig. 10) the control tumors were somewhat larger at the final charting than those of the treated animals, but the average difference was no greater than the difference existing at the time of beginning treatment after the second charting, or seventeen days after inoculation.

In the other experiments with tumor No. 180, the difference between the treated and the control tumors at the final charting was slightly in favor of the former. This difference was

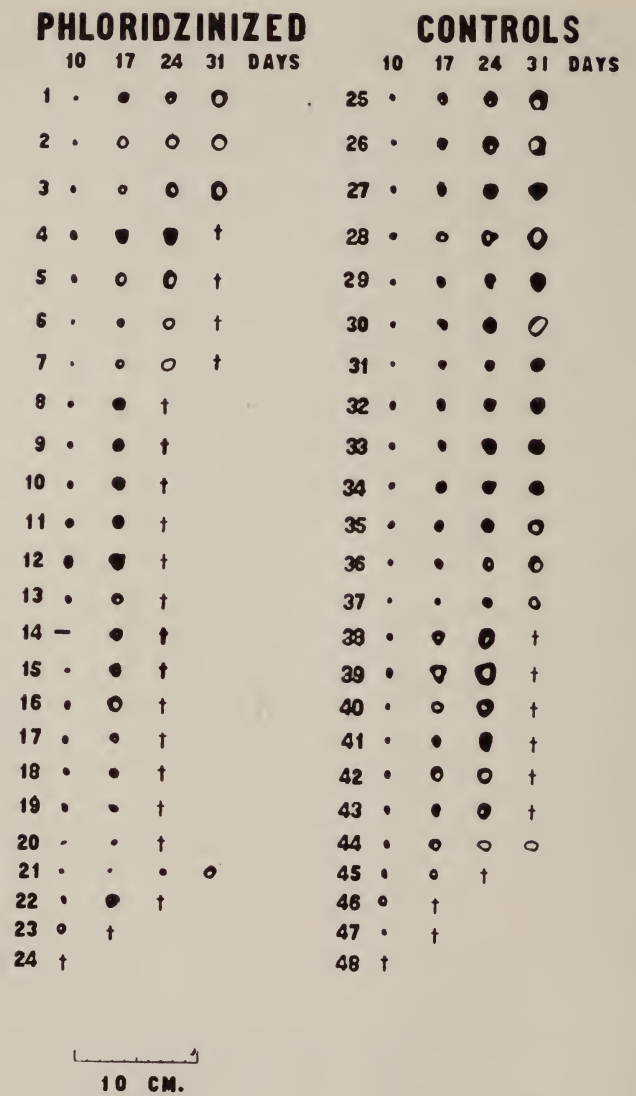


FIG. 14. Experiment $\frac{180}{5\,Q}$

On January 18, 1915, 48 mice were inoculated in right axilla with 0.01 gm. by needle method. On February 4, mice 1 to 24 were started on injections of 0.001 gm. phloridzin in left axilla. Controls: mice 25 to 48. Only eight treated animals survived one week after beginning treatment as compared with twenty of the controls. The control tumors are slightly larger than the treated.

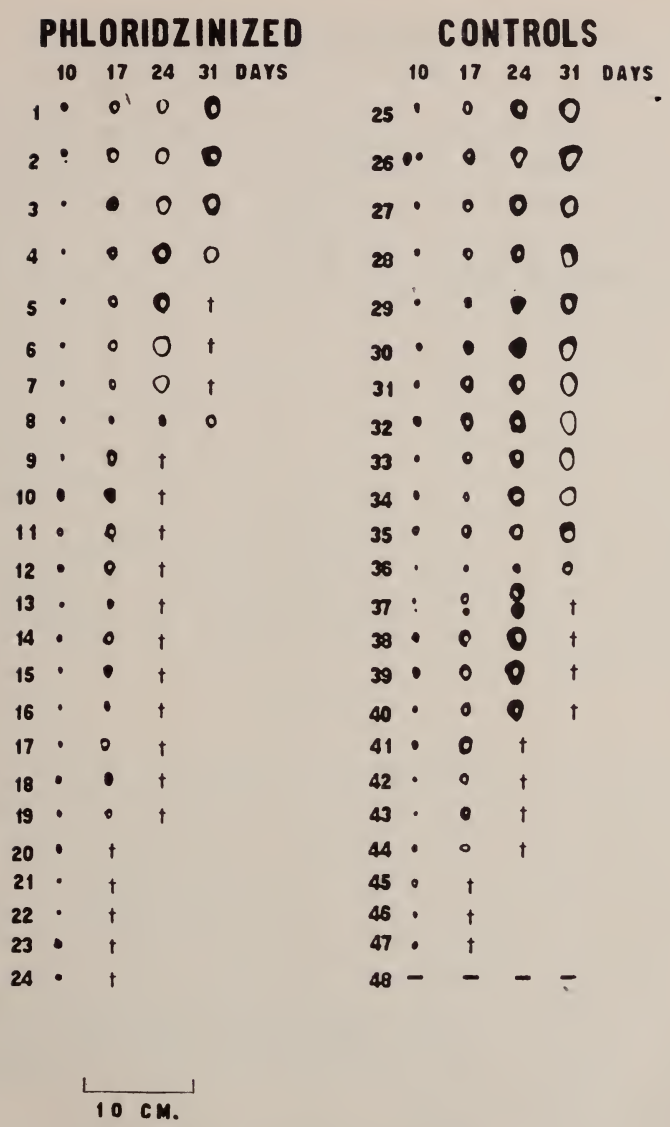


FIG. 15. Experiment $\frac{180}{5\,T}$

On January 21, 1915, 48 mice were inoculated in right axilla with 0.01 gm. by needle method. On February 1, mice 1 to 24 were started on injections of 0.001 gm. phloridzin in left axilla. Controls: mice 25 to 48. Of the tumors surviving until the third charting there is little difference in size between the two groups. The controls average slightly larger.

so small that no conclusion can be drawn either way. There was no case of "cure," or even of decrease in the size of the tumor.

Since the treatment had no effect upon transplantable growths, there was little reason to suppose that it would influence spontaneous neoplasms; nevertheless, in order to complete the experiment, a series of mice bearing spontaneous tumors was injected with phloridzin. The silhouettes of these growths are shown in figure 16, the arrows marking the time at which treatment was begun in each case. In only two animals was there any decrease in the size of the tumor, and this was no more than can be seen to occur from time to time in the natural course of growth of spontaneous tumors, from hemorrhage or from less easily appreciable causes. No. 1 in this group showed before treatment variations in size greater than those occurring in any of these tumors following treatment.

The tumors of treated animals dying or killed during the experiment were examined microscopically. No difference in morphology could be detected between the treated and the control tumors.

## SUMMARY

Among the mice bearing spontaneous tumors and Crocker Fund mouse sarcoma No. 180, there were no cases of absorption of the tumors under treatment. The slightly slower growth occurring in some of the treated animals bearing No. 180 can not be considered as due to the treatment, as the difference was not so great as often occurs in untreated animals from the normal variability of growth of this tumor. Ulceration, which is also more frequent among the treated animals, probably on account of the poor nutrition of the host, must be considered as a factor when comparison is made between the size of the treated and of the control tumors.

The Buffalo rat sarcoma showed a much smaller percentage of absorption among the treated animals than among the controls, 37 per cent as compared with 58.4 per cent. In the majority of the experiments the growth among the treated animals was much more vigorous than that among the controls.

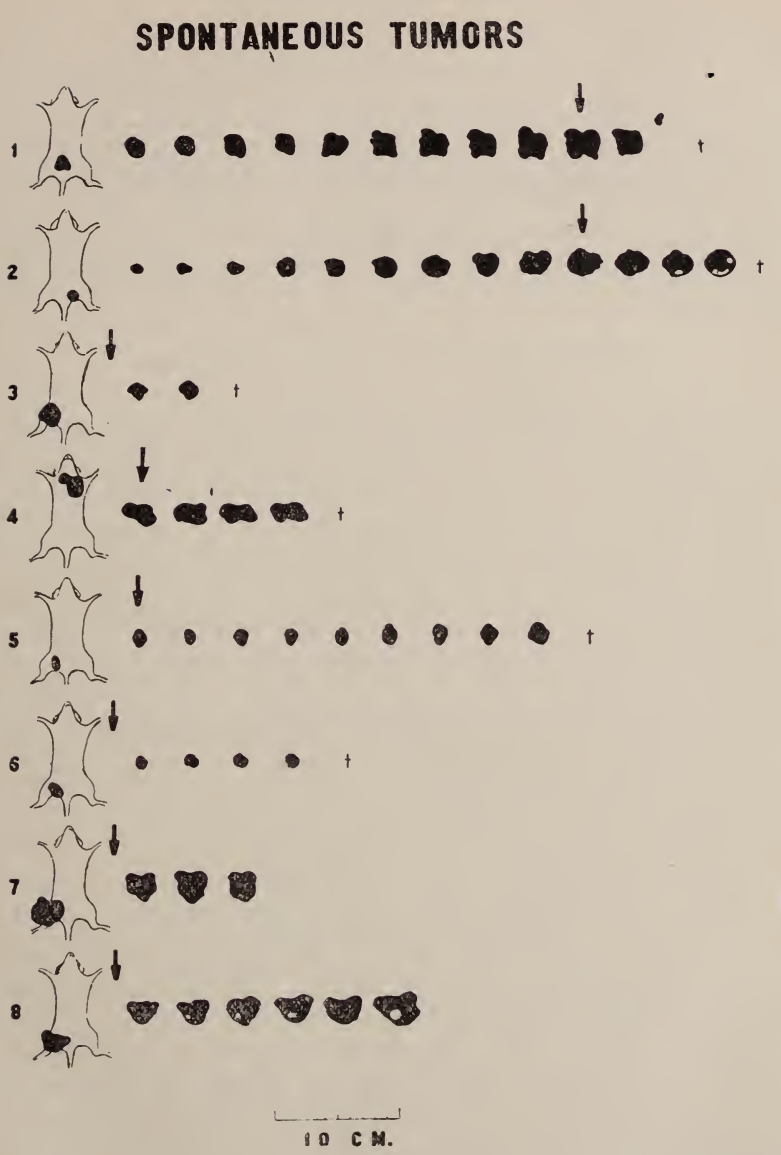


FIG. 16

Spontaneous tumors. The arrows mark the stage in the growth of the tumor at which treatment was begun.

Considering the very great variability of growth of the Buffalo rat sarcoma, as well as the high percentage of cases of spontaneous absorption occurring constantly, but with great irregularity, in different series of animals, the futility of using this tumor for therapeutic experiments or of basing conclusions upon such investigations, is at once evident. Any "cures" obtained in work with the Buffalo rat sarcoma must be ascribed to spontaneous absorption rather than to the effect of the therapeutic agent.

# PATHOLOGICAL ASPECTS OF SOME PROBLEMS OF EXPERIMENTAL CANCER RESEARCH[1]

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Although the great possibilities presented by the study of transmissible tumors of lower animals were fully revealed in 1894 by the systematic observations of Morau, it was not until 1901–2 that the work of Loeb in America and Jensen in Denmark attracted universal attention to this field. Especially in the last decade a great number of observations from a host of workers have produced a body of new data which is of first importance in oncology. From this extensive field it is my purpose to select for discussion certain problems which are of special interest to the general pathologist and to attempt to evaluate the new contributions by the old and established pathological criteria.

Prominent among the questions raised by the study of transmissible tumors of lower animals is whether these processes are genuine neoplasms. Considering that Virchow once said that no one even under torture could state exactly what a tumor is, this question must appear somewhat academic. Yet to the older pathologists, thoroughly saturated with the conviction that a neoplasm is viable only in its host, the demonstration of successful transfer to a new host naturally raised a lively scepticism. Transmissibility implied to them infectiousness and a granulomatous nature; the transplantable tumors must be shown to grow solely from the transplanted cells, after the manner of metastatic tumors, they must exhibit infiltrative growth, and

[1] Read in the Symposium on Cancer Research of the Second Pan-American Scientific Congress, Washington, D. C., January 5, 1916.

produce metastases; the striking morphological resemblance to tumors was not universally regarded as decisive evidence. All these essential attributes of true malignant tumors have now been demonstrated for many of the transplantable growths in lower animals.

Personally, I consider the morphology alone as a sufficient guarantee of the neoplastic nature of many of these tumors. Doubts of its significance seem to result from a somewhat narrow conception of the very variable morphology of the cancer process, and similar doubts have arisen regarding the cancerous nature of certain human tumors of peculiar character. Thus Borst discusses the question whether primary liver cell carcinoma is a true carcinoma because the infiltrating cells are mechanically forced into the hepatic capillaries and do not actively grow into them as do some tumor cells. Yet it seems somewhat arbitrary to demand that hepatic carcinomas should behave exactly as do mammary carcinomas. The hepatic carcinomas have many peculiarities of their own, but all the essential characters of malignant tumors. Similar reservations may be made regarding carcinomas of the thyroid, stomach, adrenal, and other organs, in which morphological details are not cut to exactly the same pattern. Moreover, cancer morphology does not always appear at its full development, but is progressively unfolding its potentialities.

Analysis of the structure of human cancer reveals the following features, any one of which may dominate the picture or any combination of which may be exhibited.

1. Hyperplasia, surpassing that observed in other conditions occurring in the same organ. A sliding scale is here necessary owing to the great variations in the degree of hyperplasia resulting from inflammatory and functional changes in the different organs.

2. Atypical qualities of the cells. This feature is universally recognized as perhaps the most significant criterion of a malignant process, and when sufficiently pronounced may stamp the process as malignant in the absence of other changes. (Early carcinoma of larynx).

3. Loss of polarity. The regular alignment of cells in relation to one another and to basement membranes may early be lost in adeno-carcinomas, and is completely destroyed in diffuse carcinomas.

4. Desmoplastic properties. The capacity to excite the growth of connective tissue is very prominent in most carcinomas, but entirely lacking in others. Mammary fibrocarcinoma and embryonal carcinoma in children represent the two extremes.

5. Infiltrative growth is a late property of most carcinomas and the time of its appearance depends much on the accidents of position.

6. Metastases. As an objective sign the occurrence of secondary tumors is most convincing evidence of a lawless growth, but in benign metastasizing stroma they occur early, in an otherwise innocent tumor, and in some types of malignant hepatoma they never develop.

Judged by these main criteria, the tumors of lower animals take their places as malignant neoplasms, with certain peculiarities which are impressed upon them by their species.

Metastases were observed and experimentally produced by Morau, and Murray found them in 50 per cent of his mouse tumors. Their relative infrequency as compared with those of human tumors may be referred largely to the short duration, and the peculiarities of the circulation in small animals. Infiltrative growth is frequently observed under suitable conditions, especially when the tumor meets resisting structures. The degree of cellular hyperplasia may be so great that the tumor outweighs the host, and in general it probably attains a larger relative volume than in human tumors. The atypical qualities of the cells are almost constantly pronounced, but while this feature has been emphasized by some, I have gained the impression that the variations in tumor morphology in lower animals are less violent and less extensive than in the human subject. Extensive overgrowth of connective tissue is also much less notable in the small animals for reasons which are not entirely clear.

I have extended this analysis to some length because the study

of lower animal tumors has forced the pathologist to relax to some extent certain rigid notions regarding what a tumor may do and how it may look.

The comparative studies have not, however, revealed any striking variations or new morphological properties beyond those occasionally exhibited by human tumors. The same fixity of form and clinical behavior reappear in the different examples of the same type of tumor, and this morphology is usually maintained throughout many generations of transplants. The structure may vary in different portions of the same tumor, and metastases may be more or less atypical than the original growth.

There is one important phenomenon exhibited by lower animal tumors, however, which is at variance with the rules deduced from human oncology. Certain transplanted tumors of mice and rats are said to excite a neoplastic process in the stroma of the host so that in the course of transplantation a stroma-born sarcoma may arise and even outgrow and eventually eliminate the original carcinoma. This phenomenon was first observed by Ehrlich and Apolant in the tenth generation of an adenocarcinoma of the mouse, and has been reported in both rats and mice by Loeb, Lewin, Bashford, Haaland, Russell, and others. The change appears to occur rather suddenly in the 8th to 10th or later generations. Russell fixed the usual period at the 55th to 60th day of propagation and states that it is independent of the number of transfers.

When once established it is usually progressive and both tumors persist together, or separate strains of sarcoma and carcinoma are obtained in subsequent transplants. The rate of growth of the mixed tumors is usually increased and the sarcoma is usually more active than the carcinoma, and is more frequently encountered in metastases (Haaland, Clunet).

The idea that the original growths were mixed tumors was abandoned for lack of any evidence. It is generally concluded that the sarcoma represents a neoplastic transformation of the stroma of the host caused by a stimulation of cells by the tumor epithelium. Both Haaland and Russell describe in detail the appearance of foci of overcellular stroma located in the centre

of carcinoma nodules, the increase in mitotic figures in the stroma cells, the survival of these altered cells in grafts, and their rapid increase until they overgrow the epithelial elements.

There is no counterpart in human pathology for this remarkable process. In no case has the stroma of a human tumor during vicissitudes of prolonged growth, inflammatory reaction from infection, recurrence after operation, or spontaneous metastases, taken on a neoplastic growth. It is true that very notable grades of reactive hyperplasia may be excited by the invasion of cancer cells. The most remarkable is probably seen in the bone metastases of prostatic carcinoma (v. Recklinghausen). Here there is very extensive overgrowth of bone following carcinomatous invasion, and histological study does not always succeed in separating tumor cells from multiplying osteoblasts in the new tissue. The process has been regarded by some as possibly sarcomatous, and secondary osteosarcomatous metastases are said to occur in the lung (Schmorl, Fischer-Defoy, Axhausen). Yet it is not clear that the osteoblasts take on true neoplastic properties, and the pulmonary nodules are generally regarded as osteoplastic carcinoma.

It is perhaps unreasonable to expect that there should be a human parallel, for human tumors do not experience quite the same insults as transplanted tumors receive. Yet it is a well established rule that the stroma never participates in the lawless growth of cancer cells except in mixed tumors, and it is distinctly anomalous that no trace of this sarcomatous transformation appears in man, when it is relatively common in mice. Under these circumstances it may reasonably be demanded that entirely demonstrative evidence should be furnished before its occurrence in mice can be accepted. Not having actively engaged in this study I am not in a position to deny the claim of the very competent investigators on whose evidence the interpretation rests, but it may be permissible to point out some of the difficulties in the way of accepting their interpretation that the stroma of mouse cancer becomes sarcomatous.

Human carcinoma never exhibits a sarcomatous transformation of its stroma, but its polyhedral cells frequently lose all

their epithelial characters and grow in spindle form. Epithelial tumors may from their inception appear like spindle cell sarcoma, as in the spindle cell basal-cell carcinomas of Krompecher, in spindle cell carcinoma of the thyroid, and in melanoma. It is becoming more and more apparent that many so-called sarcomas of the organs are in reality spindle cell carcinomas. Or the change to spindle cells may occur in one portion of a typical carcinoma as is frequently seen in tumors of the thyroid gland, and liver; or spindle epithelium may regularly appear with columnar, as in glandular adamantinoma. This tumor is notorious for its remarkable changes of cell form. In recurrences after operation on typical carcinomas, one frequently encounters pure spindle cell growths. I have traced an adamantinoma recurring after four operations, through the structures of adult acanthoma, plexiform epithelioma without squamous cells, spindle cell sarcoma, and finally round cell sarcoma. Recently I have found spindle cell perivascular sarcoma in a uterus, removed shortly after curettage, revealing typical adenoma.

Spindle tumor cells are so common in carcinoma that their occurrence in any carcinoma is very strong presumptive evidence that they are altered epithelium.

The intrinsic evidence relating to the transformation of the stroma is not entirely satisfactory. The change is sudden and coincident with an increased rate of growth of the tumor, as is the case with human tumors assuming the spindle cell form. A new tumor process, affecting the stroma, might be expected to develop more gradually. The new tumor process seems to outstrip and may eliminate the old. This observation, that an original carcinoma should yield its powers to the stroma cells and itself retire from the scene, seems highly paradoxical. The reverse process, viz., the elimination of spindle cells in the course of transplantation of a carcinoma has been observed by Apolant, but here the spindle cells were interpreted as altered epithelium. The fact reported by Haaland, that the carcinomatous element in the mixed tumor may be eliminated by heating to 44°C., is a suspicious circumstance and indicates that the spindle cells

are merely the more resistant as well as the more rapidly growing form of epithelium.

The crucial evidence is that presented by observations tracing the actual transformation of stroma cells into the spindle cell sarcoma. Not all authors have been able to convince themselves that the stroma really gives origin to the sarcoma but Russell and Haaland have traced this origin in detail. Haaland pictures peculiar halos of large pale cells surrounding epithelial groups. They are intermediate in form between stroma and tumor cells but Haaland derives them from the former. Yet the interpretation of transitional pictures is notoriously hazardous and few observers have been willing to trust it in this connection. Orth thought that Lewin's sarcoma represented granulation tissue. Through the kindness of Woglom, I have examined two cases purporting to show the sarcomatous transformation of stroma cells, but have been forced to draw from these sections the opposite conclusion, namely, that the spindle cells are derivatives of the epithelium. In view of all these difficulties it may be urged that further evidence is required before the sarcomatous transformation of mouse carcinoma can be accepted as proven. The writer would not deny its occurrence but merely asks for more evidence.

The experimental studies have not succeeded in defining what constitutes a tumor. They have shown indeed that perhaps the most essential property of tumor cells is the capacity for unlimited growth in a wide range of environments. A vast number of experiments in the transfer of normal or proliferating cells of embryonal or adult type has shown that these cells may multiply for a short period but soon differentiate and become quiescent, or atrophy and disappear. Transplanted tumor cells, however, continue to proliferate beyond the life-time of the host from which they were derived.

Yet just this conspicuous tumor character is exhibited in a notable degree by two diseases the exact nature of which has not been fully determined—the so-called infectious lymphosarcoma of dogs, and Rous' chicken sarcoma.

The infectious lymphosarcoma of dogs is transferred by coitus and also by other methods of contact. It affects primarily the genital organs, skin, kidneys and some other organs, and produces bulky primary tumors as well as miliary or massive metastases. The gross anatomy is typical of a malignant neoplasm, and the microscopical structure also is that of an extremely active, atypical, infiltrative, malignant tumor. It probably arises from the reticulum cell of the lymph-node. No micro-organisms are demonstrable in unulcerated tumors. Transplantation is successful only under the same conditions as govern the transfer of mouse tumors, i.e., the inoculation of living cells into closely related animals. Doubts regarding its true neoplastic nature arise from its evident relation to the lymphocytomas. Furthermore, it must be admitted that at some point in the history of its development a parasite may enter, though there is no evidence that a parasite persists throughout the disease. Bashford concluded that the tumor grafts grew in part at least from the host's cells like a granuloma. In serial transplants studied by Beebe and the writer it grew exclusively from the transferred cells. Bashford, on examining these sections, admitted that they differed from his own, and I am forced to conclude that he was dealing with infected material or that some other factor caused unnecessary inflammatory reaction about the grafts.

v. Dungern claims to have dealt the "coup de grace" to this tumor by showing, through the absence of agglutinins for dog's corpuscles, that the tumors lose their dog protein when transferred to foxes and accumulate only fox protein. His hare sarcoma, which had likewise certain granulomatous features, preserved its hare protein while growing in rabbits. I must decline to accept v. Dungern's conclusions, based as they are on such evidence as agglutination and hemolysis. The tumor can be seen to grow from the transplanted cells, and in comparison with such direct evidence the significance of antibodies for a specific protein is of quite subordinate value. Moreover it appears reasonable that a few dog tumor cells multiplying many thousand times in fox tissue should rapidly acquire the fox stamp. Beebe has shown that mammary carcinoma growing in

lymph-nodes acquires the nucleo-histon of lymph-nodes which is entirely missing in primary tumors of the breast.

I think therefore that the lymphosarcoma of dogs may safely be regarded as a disease sui generis, but essentially neoplastic. It may be complicated by a parasite, but the existence of this complication is not proven. Exactly the same uncertainties surround the origin and nature of human lymphosarcoma.

Rous' series of chicken sarcomas is one of the most interesting and obscure developments of experimental cancer research.

On morphology, one would accept his original tumor as a neoplasm. It presents excessive hyperplasia so far as may be judged by available standards. The atypical qualities of the cells are pronounced. There is infiltrative growth and metastases are ubiquitous. It was at first transferred with considerable difficulty and only to the same strain of chickens, but its viability gradually increased. Yet it violates the central criterion of tumor growth by being transmissible by means of tumor filtrate passed through a Berkfeld filter (No. 5 medium). The active agent will not, however, pass a Chamberland bougie F. Tumor tissue dried for some months remains active, but the resulting tumors are feeble. Exposure to 55°C. inactivates the agent. Cultures are negative.

On these data one must either discard the process from the group of neoplasms or alter the experimental criteria. The latter course seems the only logical choice.

In attempting to analyze the significance of this tumor we are handicapped because the principles of avian pathology are not well understood. One must hesitate to apply the standards of human pathology to the tissue reactions of the chicken. Exudative inflammation, functional and inflammatory overgrowth, metaplasia, etc., may follow somewhat different laws or standards in such widely separated species. Hence one may be in error in too closely identifying this chicken sarcoma with sarcomas in other animals. It may be an infectious granuloma with neoplastic morphology. But I do not think that either the granulomatous or the infectious nature is probable. The only infectious agent meeting the requirements would be an extremely

labile protozoön, of the actual presence of which there is no tangible evidence. It has been suggested as a last resort that fragments of cell nuclei capable of reproducing the cells may pass through a Berkfeld filter. While the data are still inadequate to force any conclusion, I have received the impression that Rous' sarcoma is a genuine neoplasm, occurring only in the chicken, and that the transmissible virus is of chemical, and possibly of ferment nature. The extensive series of transplants has probably intensified the action of any such chemical agent present in the original tumor, so that effects are now being produced with this tumor which were not possible with the spontaneous growth and which probably have no counterpart in any other process spontaneously occurring in Nature. At any rate, the principles deduced from this process must for the present be applied to this disease and to no other.

There are, however, some indications that chemical agents such as may be active in the chicken sarcoma, are also of influence in some human tumors. While many tumors, after their area of origin is defined, grow exclusively from their own resources, others grow by progressive inclusion of previously normal cells in the tumor sources. This principle comes to light in many different circumstances, and may be employed to account for diffusely spreading or multiple tumors of serous, mucous, or cutaneous surfaces, and in systemic tumors of lymph-nodes. In a lesser degree, it may account for collateral hyperplasia about the edges of some tumors. Paget's disease covering much of the chest and trunk would be explained thereby, and the pigment of melanoma is said to have excited the neoplastic proliferation of liver cells about hepatic metastases. So one may conceive that in the chicken tumor a chemical agent may exist which is remarkably effective in inducing neoplastic hyperplasia, and that either this agent alone or the cells that contain it may give rise to the tumor. It may here be recalled that the filtrate is much less effective than are the living tumor cells. Perhaps, also, chicken tissues are more responsive to such agents than are other animal cells.

It would be interesting to know if carcinoma in the chicken,

of which Ehrenreich has described several, is also transmissible by means of its filtrate.

In determining the effects of changes in the soil upon the growth of the tumor, experimental studies have yielded results of much practical value. Spontaneous variations in the growth of tumors are quite as well established clinically as in the experimental field, but the latter seems to have revealed some of the reasons. Racial differences in susceptibility have been practically identified with changes in the diet. The importance of carbohydrates in the nutrition of tumors has long been recognized among clinical writers and has been extensively discussed by Brault, Keating-Hart, and many others. The varying glycogen content of different human tumors suggests the caution that all tumors may not be especially dependent upon this class of food stuff, and the complexities of carbohydrate metabolism indicate that it may be difficult to secure satisfactory experimental evidence in the field of tumor diets. Nevertheless it appears that rats may be made refractory to the Buffalo sarcoma by a previous course of carbohydrate-free diet, that the course of this tumor once established is retarded by such diet, whereas it is accelerated by butyrates among the fats. Benedict saw the complete regression of large sarcomas in rats rendered completely diabetic by phloridzin. By substituting foods rich in lime, Sweet, Corson-White and Saxon rendered rats markedly insusceptible to sarcoma, and saw much acceleration in growth on return to normal diet. Contrary observations show that all tumors do not react to dietary changes, but the important feature of this work consists in the demonstration that the subject is susceptible of experimental study and opens up one of the most attractive fields in experimental cancer research.

Age is found to have no definite influence on the susceptibility to tumor grafts. Both young and old animals have proven suitable for experimental propagation, although most workers prefer quite young subjects. This rule, which could hardly have been established except by experimental studies, has an important bearing on our conceptions of the relation of age to tumor incidence. Cancer has been held to be a disease of old age and yet

young animals are the better soil for growing tumor cells. Hence it is not constitutional susceptibility but the effective action of inciting factors which belong to age. The problem of the inception of cancer is quite apart from the problem of its continuous growth. Experimental studies have had little concern with the histogenesis of tumors and only to a slight extent with their general etiology. In the transplantable tumors these important questions have suffered some neglect, but work in this field has been of great value in defining the influence of age, and separating the questions of inception from that of later growth of tumors.

Immunity: Although Lauder Brunton once said that immunity will eventually be found to be a function of the liver, the serologist will doubtless claim exclusive rights in this field. The pathologist may gladly yield this territory, pointing out that serology has signally failed to produce satisfactory evidence regarding the nature of resistance to tumor growth.

Animals in which a tumor has spontaneously regressed are often actively resistant to further implantation, but the resisting factors can rarely, if ever, be transferred to susceptible animals and specific antagonistic factors in the blood serum have not been satisfactorily demonstrated. On the contrary, it is rather clearly apparent that immunity to tumors is histioid and cellular and reveals itself in the reactive growth of connective tissue, phagocytosis, and lymphocytic attack on the tumor cells. A sufficient stroma reaction has been emphasized by many as the essential element in successful implantation, but abortive grafts are often found sharply enclosed in alien connective tissue. Phagocytosis is very frequently observed about grafts implanted in insusceptible animals, and many types of cells participate in this process.

Lymphocytosis and lymphocytic invasion are prominent in the reaction against grafts in resistant animals and about regressing tumors. There have been numerous observations pointing to the importance of lymphocytes, large and small, in the local and general reaction to tumor growth. Recently Murphy has shown that mouse tumors may grow in chicken embryos until the time when the production of lymphocytes becomes es-

tablished, that this time may be shortened by implanting spleen tissue in the embryo, and that tumors may grow in alien species whose lymphocytogenic function is paralyzed by the x-ray.

Most of these observations on the mechanism of tumor immunity must be valued as confirming conclusions previously drawn from human pathology. Schmidt has shown that vagrant tumor cells are not destroyed in the circulating blood, but are lodged in capillaries, coated with fibrin, inclosed by endothelial cells, and reduced to fibrous nodules. It is highly probable that many tumor cells are destroyed in the lymph nodes which drain tumor areas. The function of lymphocytes in limiting tumor growth is extensively illustrated in human material. In many instances they form the chief barrier against the initial downward invasion of epidermoid carcinoma. In many mammary carcinomas one may see islands of tumor cells in process of destruction by lymphocytes, and polyblasts figure prominently in the active fibrosis which incarcerates many tumor cells. It is a well founded principle of pathology that degenerating or alien tissue cells are removed by phagocytosis. Reactive fibrosis limits the growth of many tumors, more often in man than in lower animals, and may be interpreted as a form of cicatricial healing. It may reach very extensive proportions; thus, I have seen nearly the whole liver transformed into scar tissue in which were very scanty remnants of an original metastatic carcinoma. Lymphocytic activity and extensive fibrosis are features that belong to the so-called clinically resistant cases, and are generally wanting in the more rapidly progressing carcinomas. These processes seem to be particularly prominent in very old subjects in whom carcinoma is of slow progress and often takes a scirrhous form. Back of these well known histological signs of the mechanism of immunity are doubtless submerged constitutional and local influences, in the elucidation of which tumor serology doubtless faces a significant future.

On the therapeutic side, experimental cancer research still presents itself practically empty-handed. The exclusive and quite energetic pursuit of the principles of serum immunity has accomplished practically nothing, except to show that the malig-

nant tumor process can probably not be controlled by investigations along the lines which have proven effective in bacterial diseases. Vaccination by means of tumor derivatives has been practised on an enormous scale all over the world and has failed to justify itself. It has had some paradoxical success but has probably done more harm than good. No one has been able to improve upon Vidal's feeble showing for anticancer sera. Chemotherapy has never had any tangible basis in the tumor field, and from Weil's review it would seem to have even less claim to serious notice than has vaccination. Under these circumstances, I venture to raise the question whether it is not time to abandon this unprofitable territory and seek for help in entirely different directions. It may be that artificial alterations in the course of metabolic processes in the body, as suggested by recent dietary studies, may prove capable of influencing favorably the course of some malignant tumors. I freely confess the hope that the vegetable kingdom may be found to contain some agent that will specifically affect the cells of some tumors. It is the genius of vegetable products specifically and powerfully to affect different organs, tissues, and functions of the animal body, as exhibited by digitalis, strychnine, morphine, etc. Why should not some vegetable agent attack the delicately balanced nutrition of tumor cells? It would be extremely disconcerting, and even mortifying, if some vegetable alkaloid or glucoside were found to do more for cancer than all the theories of serum immunity, but the demonstration, if made, would have to be accepted.

At present, the only laurels in cancer therapy are being carried off by physical agents, x-ray and radium, and it seems to be only the difficulties of accessibility and dosage which stand in the way of the successful application of these agents to all localized and some generalized tumors. Cancer research should note that progress in the development of the x-ray is almost exclusively in the hands of elaborately equipped and far sighted business corporations, from whom one department of medicine receives orders when and how to proceed. This situation is not flattering to our dignity. With radium, the situation is somewhat different, since the study of the physics and therapeutics of

radium rests with Government organizations, universities, and private institutions endowed with a supply of this precious metal. It is not too much to hope that when the early stages of cancer are recognized as a proper field for the use of x-ray and radium, as has already occurred in the opinion of some competent authorities, much of the present fear of the disease, especially of the most deplorable post-operative recurrences, will be removed. In such an event, some of the present problems of cancer research will retain only an academic interest.

In the department of special etiology, experimental cancer research has secured some of its most significant results.

The chicken sarcomas are most suggestive from the point of view of their probable etiology and pathogenesis. Although there may be nothing like them in human pathology, they stand as a specific pathological entity and raise interesting questions regarding the etiology of other tumors. Borrel's consistent pursuit of animal parasites in tumors has enabled him and others to uncover the main factors in the causation of several tumors of lower animals and it is not impossible, although as yet unproved, that they may have counterparts in man. When Fibiger discovered a nematode worm in the gastric carcinoma of rats, and by a brilliant analysis identified and located this parasite in Nature and reproduced the disease experimentally, he established the existence of another specific disease of neoplastic character.

These contributions seem to me to point to the necessity of regarding all forms of neoplasms as specific diseases, connected only by the fact that they are neoplastic in greater or less degree, but differing in their etiology, clinical course, and therapeutic possibilities. In the same way, tuberculosis and bubonic plague are infectious diseases of inflammatory nature, but they are quite as closely related as Fibiger's gastric carcinoma of rats and pipe smokers' cancer of the lip. The habit of regarding cancer as a protean disease of uniform significance may well be abandoned in the interests of progress. When cancer research properly occupies itself in the study of the distinctive features of different cases of malignant disease, especially when it abandons the idea of a

universal cure for cancer, it will be in accord with sound pathological sense. It will then not be necessary to talk wisely to the public about the obscurities of cancer etiology, or speculate about why cells grow lawlessly. Concerning the ultimate nature of neoplastic overgrowth we shall never have more than a descriptive knowledge.

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# TRANSPLANTABLE SARCOMATA OF THE RAT LIVER ARISING IN THE WALLS OF PARASITIC CYSTS

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An earlier paper[1] recorded the occurrence of a primary sarcoma in the wall of a cestode cyst of the liver of a rat. The malignant nature of this small tumor was not recognized in the gross and consequently no attempt was made to propagate the growth.[2] The present report presents two tumors of the liver of rats, arising from the cyst wall of a Tenia crassicola, but differing from the first in their extensive growth and widespread metastases. As the gross appearance of these neoplasms was diagnostic of malignancy, the tumors were transplanted.

*Crocker rat sarcoma No. 7.* The host was a full grown female white rat of unknown age. Upon opening the abdomen a mass measuring about 2 x 3 cm. was found near the left border of the right lobe of the liver. Section through this mass showed an infiltrating growth with a central yellowish core. The pancreas, which was not adherent to the tumor, contained numerous small secondary nodules, chiefly at the splenic end. The lower third of the spleen was replaced by a large metastasis. The right kidney was represented by a large necrotic mass. The mesentery and the peritoneal surface of the small intestine and colon were studded with numerous small metastatic nodules. The omentum was almost entirely replaced by neoplastic tissue, and metastases were found also in the diaphragm and in the tissues

[1] Bullock, F. D., and Rohdenburg, G. L.: Jour. Med. Research, 1913, xxviii, 477.

[2] Similar tumors have been recorded by Borrel (Bull. Inst. Pasteur, 1907, v, 497), Bridré (Compt. rend. Soc. Biol., 1909, lxvi, 376), and others.

about the ovaries and the adrenal glands. No gross metastases were demonstrable in the lungs or in the lymph nodes outside the peritoneal cavity.

Histologically the hepatic tumor was a small spindle-cell sarcoma, composed of cells of irregular size, many of which showed mitotic figures (fig. 1). The center of the tumor contained a dead parasite (fig. 2) surrounded by necrotic tissue. In certain portions of the neoplasm isolated strands of liver cells and bile

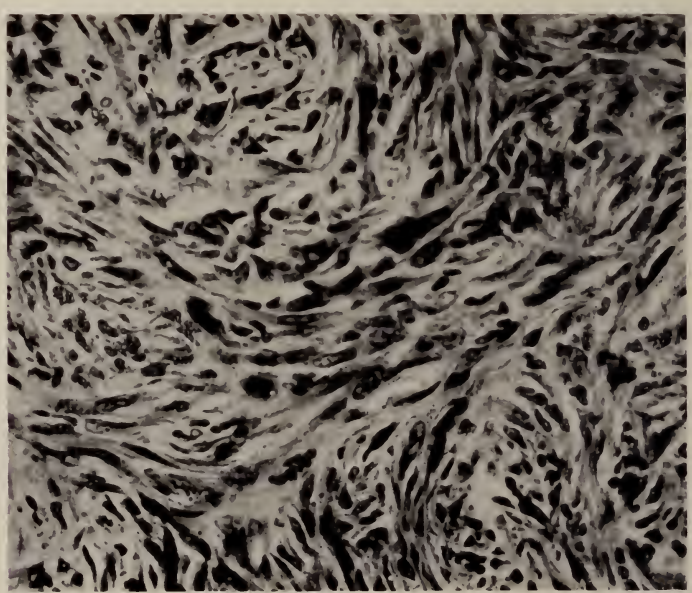

FIG. 1. Sarcoma in wall of cyst about a parasitic worm. × 250.

ducts were abundant; in still other areas the tumor was exceedingly vascular. The secondary deposits in the various organs (fig. 3) were identical in structure with the hepatic tumor.

The site of origin of the growth is indicated by the central location of the parasite and the surrounding necrosis.

Cultures of the necrotic material replacing the right kidney were sterile.

The tumor on transplantation behaved like other transplantable rat sarcomata. As will be noted in the appended Table I,

the inoculation percentage of the tumor is high and growth is rapid, although the majority of the tumors undergo spontaneous absorption. The growth is now in the 10th generation, with an inoculation percentage of 100 and an absorption percentage

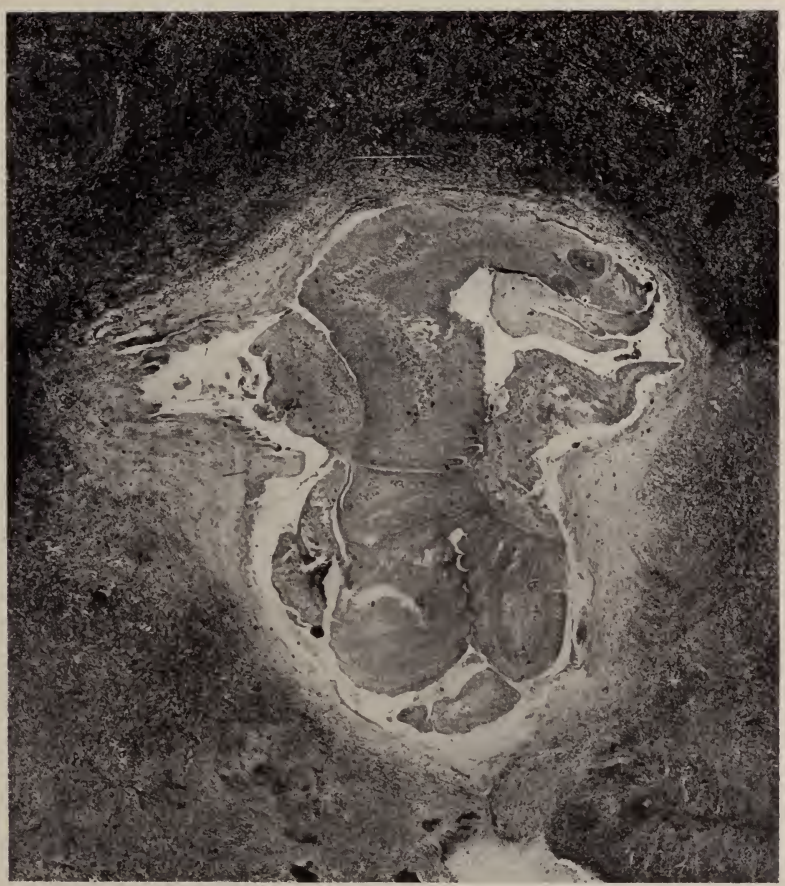

FIG. 2. Parasitic worm in liver surrounded by sarcomatous tissue. × 25.

of 95. An attempt was made to propagate the tumor, using dried tumor powder and a Berkefeld filtrate after the method of Rous. The outcome of the experiments, however, was negative.

*Crocker rat sarcoma No. 8.* A full grown white male rat was the bearer of this tumor. The primary growth occupied the

upper and lower poles and the upper posterior wall of a rather large cyst which was attached to the Spigelian lobe of the liver by a short, broad pedicle. The liver itself apparently was only slightly involved in the malignant process. The mass, consisting of cyst and tumor tissue (fig. 4), was ovoid in shape, somewhat nodular on its anterior and posterior surfaces, and measured 1.5 x 1.5 x 2 cm. Cross section, after fixation, showed that the upper pole and a part of the cyst wall were replaced by tumor tissue which encroached upon the cyst cavity, and formed the somewhat nodular surface of the cyst. The growth in the upper pole measured 1 cm. at its thickest portion. A part of the lower pole of the cyst was composed of tumor tissue and had a maximum

TABLE I

| NO. OF GENERATION | NO. INOCULATED | PERCENTAGE TAKES | PERCENTAGE SPONTANEOUS DISAPPEARANCE |
|---|---|---|---|
| | | *per cent* | *per cent* |
| 1 | 365 | 70 | 89 |
| 2 A | 36 | 100 | 77 |
| 2 B | 36 | 100 | 66 |
| 2 C | 48 | 75 | 65 |
| 3 A | 36 | 79 | 69 |
| 3 B | 36 | 80 | 75 |

thickness of 3 mm. The portions of the cyst wall lying between the tumors were apparently slightly thicker than the usual thickness of these cysts. The cyst cavity was filled with a large cestode worm.

Many small, round, secondary nodules were found scattered throughout the omentum, mesentery, and upon the serous coats of the small intestine; the omental metastases were by far the most abundant. The diaphragm contained a small tumor, but the thoracic viscera showed no gross metastasis.

Microscopically (fig. 5) the upper tumor was composed of a rather compact mass of cells which, in general, showed no definite arrangement, although in particular areas the cells tended to form in parallel rows or in groups. Variations in size and polymorphism were characteristic features of the cells. They were

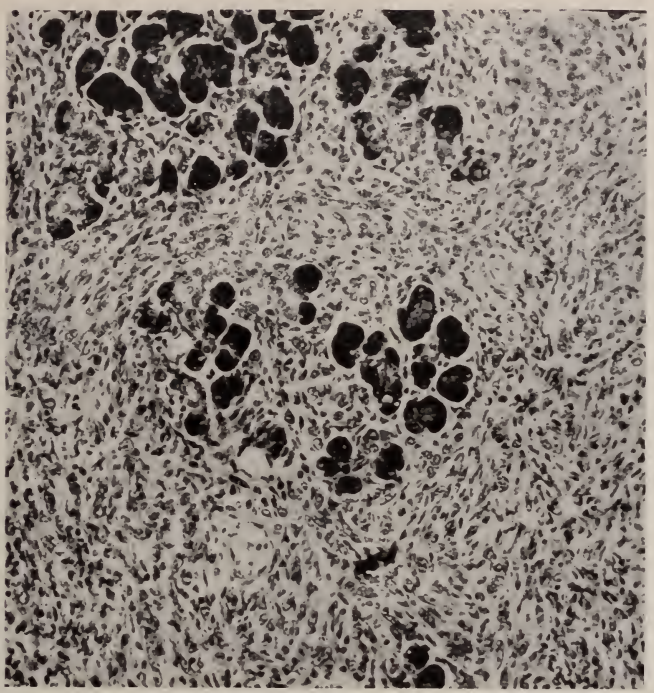

FIG. 3. Metastatic nodule of sarcoma in splenic portion of pancreas. × 150.

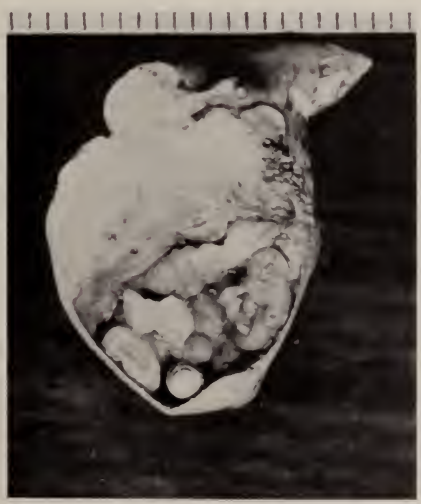

FIG. 4. Gross appearance of Crocker rat sarcoma No. 8. Scale in millimeters.

round, polygonal, sometimes oval or spindle in shape, and possessed an indistinct outline. The nuclei showed a corresponding variability in shape and size, and mitotic figures were numerous. Scattered freely through the tumor were many small and large multinucleated cells. The scanty stroma of the tumor consisted mainly of fine spindle cells with a few delicate connective tissue

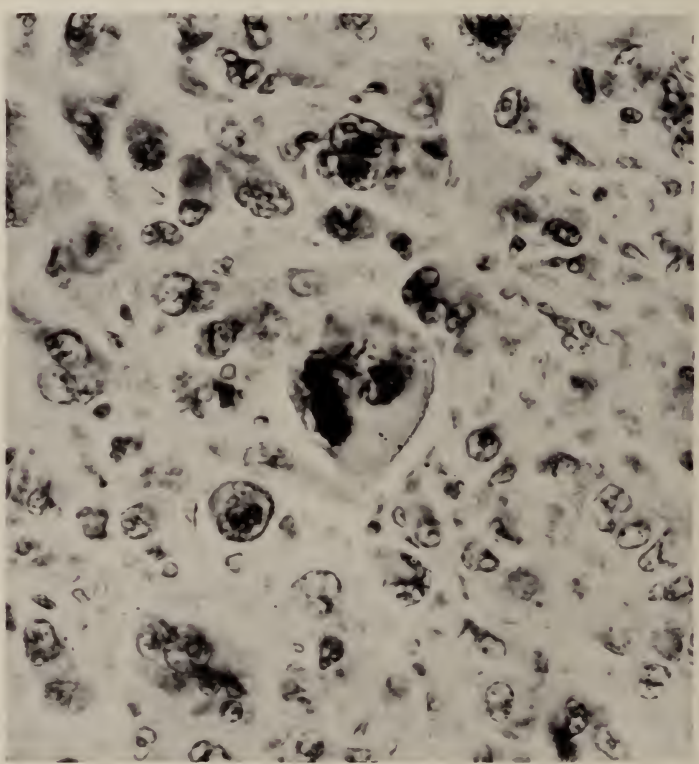

FIG. 5. Microscopical appearance of tumor at lower pole.

fibrils. The tumor was richly supplied with blood furnished in large part by capillaries which were especially abundant where the cells were arranged in rows, and here apparently supplied the only stroma. Other tumor cells arranged in groups were partially or completely surrounded by blood capillaries. Degenerative changes and hemorrhage were observed in certain

areas. A few bile ducts and an occasional liver cell were found in the portion of the tumor bordering the cyst cavity.

The lower tumor differed from the upper in the preponderance of giant cells, in the greater degree of cell degeneration, and in an utter lack of any definite cell arrangement.

FIG. 6. Cyst showing tumors at upper and lower pole and the relation of the tenia. Low power.

The cyst wall between the upper and lower tumors was very vascular and contained many cells, chiefly of the spindle type.

Eosinophiles were numerous. Giant cells and cells resembling those of the tumor were found in the cyst wall at a remote distance from the tumor.

Lining the cyst cavity were cylindrical cells showing degenerative changes, which reached the stage of complete necrosis where they lay beneath the tumors.

The metastatic tumors presented the general appearance of the primary growth except that the cells showed more regularity in shape and size, and fewer giant cells were found.

The tumor on transplantation differed from tumor No. 7 in the smaller percentage of takes.

The good staining qualities of the tissues of the tenia (fig. 6) is an indication that the parasite was alive when the tumor was discovered; this fact was not noted before as the specimen was fixed in toto.

These two cases of the association of parasite and tumor are not presented as an argument in favor of the parasitic hypothesis of the origin of malignant neoplasms, but rather as additional examples of malignant tumors following chronic irritation.

# CHEMOTHERAPEUTIC EXPERIMENTS ON RAT TUMORS[1]

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In discussing a subject of such extent as the chemotherapy of tumors it is quite evident that only isolated features of the problem can be touched on. In the present paper I shall attempt to discuss certain phases of the work which I have been carrying on for several years, in their bearing on the general problem.

1. *The penetrability of the living tumor cells.* There is at the present time considerable unanimity on the subject of the intravitam staining of cells. Goldmann (3), who was one of the first to study the distribution in the cells and tissues of dyes which were introduced into the circulation, reached certain conclusions which have largely served as the point of departure for subsequent study. He found that certain of the cells took up these dyes, which could then be identified as characteristically colored granules distributed through the cells. Goldmann considered that these granules were preformed elements of cell structure, which had been stained by the dyes. It is possible that this explanation holds true of certain basic dyes (such as janus green, methylene blue, neutral red). Upon this theory it is clear that a staining of granules is simply an alteration of paraplasm, or deuteroplasm, and that, in the absence of a diffuse staining of the cytoplasm we cannot speak of a true vital stain of the cells. Indeed, Fischel (2) in Ehrlich's Encyclopädie, holds that there is no such thing as a vital stain in the latter sense.

[1] Read in the Symposium on Cancer Research of the Second Pan-American Scientific Congress, Washington, D. C., January 5, 1916.

On the other hand, it is now widely believed that staining of preformed granules is not the correct explanation in the case of the dyes used by Goldman, which belong almost entirely to the benzidine group. Evans and Schulemann (1) have argued quite convincingly that the presence of the intracellular granules in the cells, in the case of this group of dyes, is due solely to phagocytosis. The cells take the circulating particles of dye out of the circulation, and they then appear as densely stained particles within the cell body. In harmony with this view, it is found that only certain definite types of cells, which act as phagocytes—the "scavenger cells" as Evans calls them, can take up these dyes. The living cells of tumors do not belong to this category, hence it is impossible to stain them in vivo with the benzidine dyes. Indeed, I have not been able to stain them with any dyes whatever. The cause of this differentiation between cells is probably biological rather than physical. The scavenger cells are differentiated to pick foreign substances out of the blood, for the purpose of elimination. They are, so to speak, a widely disseminated excretory organ. This view is borne out by some instructive experiments carried out by Kite (6). He found that certain dyes (such as azolitmin, congo red, tropeolin 000 No. 1, alizarin sulphonate, indigo-carmin) which fail to penetrate amoeba proteus, diffuse freely throughout the organism when injected into it. In other words, the surface of the cell offers an obstacle to its entrance; once in, the color is taken up diffusely by the protoplasm. Again, he states that "if the egg of Asterias be punctured, the acid dyes used penetrate the swollen area for varying depths, but never enter the normal unswollen protoplasm."

Consequently, it is found that dead or injured cells behave quite differently towards the benzidine dyes. Their peripheral resistance is gone, and they take up the dyes rapidly, presenting a uniform stain. Thus the cells of the kidneys of rabbits treated with sublimate or cantharidin (Gross (4)), and the anterior horn cells in experimental poliomyelitis (MacCurdy and Evans (7)) may be strikingly stained by these dyes.

In this connection, the claims of Wassermann and Keysser (10)

with reference to the staining of living tumor cells, are worthy of attention. They asserted that the eosin penetrates all the cells of the body, and therefore used it as a carrier ("cytotrochin") for selenium. Their facts, however, do not bear out this assertion. They never succeeded in staining tumors smaller than a cherry pit in size—in other words, tumors in which central necrosis had not occurred. Benign spontaneous tumors, which have no tendency to undergo necrosis, they invariably failed to stain. Internal implantations, which have an infiltrative mode of growth and are well supplied with vessels, Keysser (5) was never able to stain. It seems quite clear, therefore, that they did not succeed, as they thought, in staining the living cells, but only the necrotic areas. In a recent paper (11) I have critically analyzed these results.

2. *Staining of necrotic areas.* Inasmuch, therefore, as the conditions do not permit of staining the living cells of the tumor, one is perforce driven to a study of the staining reactions of the necrotic parts of tumors. It is this phase of the problem to which I have chiefly devoted my attention. The literature which bears on the subject is to be found almost exclusively in the remarkable series of studies which have been published in the last few years from the Sprague Memorial Institute (8) under the direction of H. G. Wells, dealing very largely with the staining reactions of tuberculous tissue.

The first question which was studied in my work concerns the distribution of crystalline substances in necrotic tumors, as compared with the normal tissues of the body. Rats bearing necrotic tumors received intravenous or subcutaneous injections of solutions of sodium iodide. After varying intervals of time the animals were killed, and the various tissues of the body were analyzed for their iodine content. The chemical work was carried on by Dr. Van Alstyne, to whom my thanks are due. It was found that the blood regularly contained the largest proportion, and after this came the tumors and the liver; the other tissues, except the kidney, have regularly shown very much less iodine. If the tumor was small and non-necrotic, its proportion of iodine was very small. These findings are entirely in harmony

with those recently published by Wells, DeWitt and Corper (12) on the distribution of potassium iodide in tuberculous tissue. They interpret their results as indicating that "the large amount of iodine present in necrotic tissues, whether tuberculous or otherwise, is dependent on purely physical conditions, i.e., the destruction of the semi-penetrability of the cells."

3. *Localization of dyes in tumors.* The relation of the dyes to the necrotic tissues of tumors is of considerable interest. Wells, DeWitt and Corper arrived at the conclusion that "necrotic tissues, whether tubercles or other lesions, behave like any non-living colloidal mass into and from which crystalloids diffuse readily and rapidly, while colloids enter very slowly or not at all." In support of this view they showed, by a very ingenious application of anaphylactic methods, that egg white does not penetrate the caseous tubercle. However, their theory does not appear to take account of all the facts. Thus Dr. DeWitt (13) herself has shown in another paper that caseous tubercles can be thoroughly penetrated and stained by trypan red and by trypan blue. Both of these dyes, however, are colloidal. As an actual fact, the relationship between foreign colloids in the circulation and necrotic tissues are very much more complex than might be inferred from the hypothesis above outlined. In illustration of this fact I might instance the following observation.

During the period in which I was studying the distribution of dyes in tumors, the rats in our laboratory fell a prey to a very serious epidemic. The disease manifested itself as a progressive caseating inflammation of the lungs. Macroscopically and microscopically the lesions presented a striking resemblance to human phthisis, without, however, showing cavity formation. Very frequently I autopsied animals in which not only necrotic tumor tissue, but also these caseous areas in the lungs were present. If such animals had previously been injected intravenously with appropriate dyes of the benzidine series, it was almost invariably possible to make a very striking observation. The dye stuff, e.g., congo red, regularly produced intense discoloration of the necrotic tissue in the tumors, but in no instance, over dozens of observations, did it produce the slightest staining

of the caseous areas in the lungs. Congo red, it may be added, is a highly colloidal dye. This statement is based on the fact that the dye does not diffuse through membranes. Certain other properties of its solutions seem, however, to range it among the highly dissociated electrolytes. Such, for instance, are the effects upon the boiling and freezing points, and the fact that its solutions are optically clear. Therefore, one may conclude that the relation of colloids, or of colloidal dyes at least, to necrotic tissues is not uniform.

As a matter of fact, the diffusibility of dyes into a colloidal mass depends upon a variety of circumstances. As regards the dye, not only its degree of diffusibility through animal membranes which determines its value as a colloid, but its electrical charge, its chemical reaction, and its chemical composition all play a rôle. As regards the colloidal mass again, its physical composition is of importance, as is also its electrical charge and its chemical composition. A knowledge of these factors permits of a fair guess, but no more, as to the result. Thus Teague and Buxton (9) found that agar, which is supposed to carry a negative charge, was easily penetrated by acid dyes, anilin blue diffusing as actively as the much more slightly colloidal eosin. Of the basic dyes, only the slightly colloidal ones diffused in any amount. Even moderately colloidal basic dyes showed little capacity to invade the agar. The basic dyes, however, stain the agar intensely, whereas the acid dyes leave it uncolored. Congo red and azo blue constitute an exception to the latter rule, and although they are acid dyes, diffuse only slightly but stain intensely. Thus it will be seen that no generally valid law for the diffusion of all colloids, or colloidal dyes, into necrotic tissues can be formulated. The same dye may react quite differently to different types of necrosis even in the same individual.

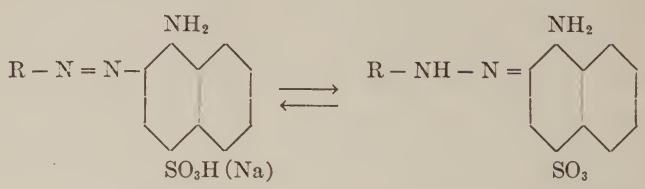
4. *Localization of the benzidine dyes.* Of the benzidine group, a considerable number of dyes have been tested, starting with congo red. In all over 20 dyes have been included in the present study, all of which are well known chemical individuals of the benzidine group, representing substitutions either in the benzene or in the naphthalene nucleus by sulphuric acid, hydroxyl,

methyl, salicylic acid, and other groups. In general these dyes tend to localize first in the peripheral zone of the central necrotic area. If repeated injections are made, they gradually penetrate the entire necrotic mass. Eventually, with the use of very large amounts, some of these dyes may slightly discolor even the healthy, actively growing rim of tumor tissue, owing to the presence therein of minute foci of necrosis. For the other tissues of the body, the stains have a varying degree of affinity, depending on the degree to which they are taken up by the scavenger cells. Superficially it would appear that certain dyes have a relatively specific tropism, if one may use this term, for the necrotic areas of the tumor. It is, however, not safe to judge of the localization of the dyes by the discoloration of the skin. Animals which have been treated by intravenous injections may on autopsy present no apparent evidence of discoloration, either in the skin or in the internal tissues. If, however, the liver be boiled for a few minutes—a procedure long ago suggested by Ehrlich—the masked discoloration at once becomes evident. Observing this precaution, I have never yet found discoloration in the tumor which was not accompanied by some discoloration in the liver, either alone or with other of the parenchymatous viscera. On the other hand, many of the dyes appear to lodge in far greater amount in the necrotic area of the tumor than in any other tissue, and also to remain there for a longer period. Whether they are actually present in greater amount there than in the liver is, however, very doubtful, owing to the fact that the underlying colors make an ocular comparison extremely fallacious. In some experiments, equal weights of liver and of necrotic tissues were suspended in equal volumes of water, and the colors of the resulting solutions were compared. It did not appear that the liver contained less than the tumors in any instance; indeed, it occasionally contained more. Thus, it is probably correct to say that none of the benzidine dyes manifests more than a relative specificity for the necrotic tumors. And it is not unlikely that even this apparent specificity is actually nothing more than the expression of a retarded rate of absorption from these poorly vascularized areas. It is interest-

ing to note that these dyes manifest the same predilection for the necrotic areas of human tumors. A solution of congo red was injected intravenously into a patient with cancer of the breast in the hope of helping in the detection of the carcinomatous areas at the subsequent operation. When the breast, with the axillary contents, was removed, it was found that the necrotic areas of the tumor foci, both in the breast and in the axillary nodes, were stained an intense red. The living areas of tumor tissue, as well as the normal tissues of the breast, appeared to have their normal coloration. It seems unlikely, however, that this method will ever prove of any clinical value.

5. *Metachromasia.* An interesting phenomenon is the changes of color undergone by some dyes after they enter the necrotic areas. This color change has been described as metachromasia, adopting the terminology of pathologists. The cause of the phenomenon is somewhat obscure. It is, of course well known that some of the benzidine dyes are markedly affected in color by the mineral acids. Indeed, congo red has on this account been adopted as an acid indicator. But the organic acids have this effect only in high concentration and in minor degree. Moreover, the color changes are not similar to those induced by mineral acids, but resemble more the changes induced by the localization of dyes in amyloid. In seeking to determine the cause of these changes I was led to test the effect of solutions of various polypeptids on the benzidine dyes. I found that they effect changes quite similar to those produced in vivo by the necrotic tissues. Thus if solutions of congo red be mixed with solutions of various amino-acids, a series of changes in color can be produced, slight, for example, in the case of glycyl-glycine, more marked with leucyl-asparagine and alanyl-glycine, and pronounced in the case of glycyl-glycyl-leucine. The color does not change to blue, as it does with mineral acids, but rather to a deep mahogany brown. Mr. Carruth has suggested to me that color changes in dyes like congo are to be explained by a separation of the base (Na) from the acid radical, which makes it possible for the dye to assume the isomeric quinone form. Although the free congo acid may exist in a red form (azoid), this

form is not stable in aqueous solutions and passes instantaneously into the blue form (quinoneimide). Intermediate shades—brown-violet, etc.—must represent the presence of certain amounts of each form:




At all events, it appears that the chemical conditions present in the necrotic tissues results in the production of some change in the character of the dye compound. In the same animal the normal tissues, such as the skin, which take up the dye, present it in an unaltered form. This fact led to the hope that the necrotic areas might conceivably be competent to break up compounds of these dyes with toxic substances, freeing the latter for attack upon the surrounding healthy tumor cells.

6. *Therapeutic effects of certain compounds.* The method of study followed in determining the effect of the dyes upon tumor growth, and a typical result, are illustrated by the following protocol. In this case the dye used was Columbia violet R, supplied by the Berlin Aniline Works. It is a diphenyl-disazo-di-amino-naphthol-sulphonic acid.

A rat, series A, X, weighing 170 grams, has a sarcoma, the Buffalo strain, inoculated 27 days previously, now measuring $\frac{1}{2}$ by $1\frac{1}{4}$ inches, firm, not ulcerated.

April 24, subcutaneous injection: 3 cc. 0.5 per cent C. v. R. No constitutional effects; no discoloration except at site of injection.

April 25, the same dose.

April 26, the same dose.

April 27, no general discoloration. Exploratory section of tumor: center necrotic, peripheral necrotic rim shows a violet discoloration. Two pieces removed from healthy margin and planted in 10 rats, in all of which "takes" occurred. Skin sewed up and soon healed.

May 11, $1\frac{1}{2}$ cc. 1 per cent C. v. R., intravenously. Tumor has grown to 1 by $2\frac{1}{2}$ inches.

May 13 and 14, 2 cc. 1 per cent, intravenously.
May 17, animal died.
Autopsy: Tumor unstained in growing margin. Necrotic core shows all transitions in color from salmon yellow at center to violet at margin. Infiltration of lungs, with severe caseation. No discoloration. Liver and kidney on boiling show slight discoloration.

A series of compounds analogous to congo red were made for me by Mr. Carruth, working under Professor Orndorff in Ithaca. In determining the compound to be made we were perforce guided by largely speculative considerations as to their probable effects upon the living cells. Unfortunately the data upon which such calculations can be based are few and inadequate. Aside from this consideration, however, it is evident that chemotherapeutic compounds, to be of any possible service in the treatment of tumors, must possess certain other properties. They should not be highly toxic to the organism. They must, of course, be soluble. They should be fairly stable in solution, yet should be dissociable in the necrotic areas of the tumors. These properties are not such as can be foretold of any given compound with certainty, hence the investigation resolved itself into an empirical study of such of the compounds as seemed most favorable.

The compounds which were tested out on the rat tumors are comprised in the following list:

*In solution*

1. Ortho-diselenide dye.
2. Congo-formaldehyde compound. No free formaldehyde present. 10 grams congo red per liter; 0.43 grams of formaldehyde per liter.
3. P′-arseno-aniline dye 1 per cent.
4. Mercury congo blue, 0.5 per cent; probably a mercuramino compound.

*In powder form*

5. Recrystallized congo red.
6. Recrystallized bordeau extra.
7. Barium salt of congo red.

8. Potassium salt of congo red.
9. Zinc salt of congo red.
10. Copper salt of congo red.
11. Copper salt of bordeau extra.
12. Congo di-triazine.
13. Benzidine < sulphanilic acid. / Beta-naphthol.
14. Thio-aniline dye.
15. Benzidine < Atoxyl / Beta-naphthol
16. Benzidine < Atoxyl / Naphthionic acid.
17. Soluble selenium congo red.
18. Salmon red, thiazol derivative.

The exact composition and probable formulae of these compounds will not be here discussed. Consideration of solubility, toxicity, and other properties will also be deferred to a future publication. The present paper is concerned only with the analysis of their therapeutic effectiveness.

All the compounds appeared, judging by the gross discoloration of the tissues, to localize electively in the necrotic areas of the tumors. All the injected animals in which tests were made, however, showed discoloration of the boiled livers. The localization was further controlled by chemical analysis of the organs which, at least in the case of the arsenic compound, could be considered to give reasonably accurate results. The maximum yield of arsenic per gram of substance was obtained from the liver, while the tumor and the kidney came next. The arsenic content of the other organs and tissues was low. From these results it would appear that localization in the tumors was only relatively specific. It is, however, possible that the arsenic reached the liver only after the compound had been dissociated in the necrotic areas of the tumors.

In judging of therapeutic effects, three criteria were employed, namely, the rate of growth of the tumors, their transplantability, and the number of retrogressions. The details of the method have already been illustrated. None of these three criteria has

an absolute value in the type of tumor which formed the basis of this study, inasmuch as they all vary to a remarkable degree. At times the tumor "takes" in a large percentage of the inoculations, while at other times, for no ascertainable reason, this percentage is greatly reduced. The rate of growth and the percentage of retrogressions also vary strikingly in different generations of the tumor. For this reason it is always necessary to plant a considerable series, of which at least half are kept as controls, while the remainder are reserved for the purposes of the experiment. The conditions are unfortunately such as to preclude the determination of small effects; on the other hand a definite and considerable influence on the life history of the tumors could certainly not escape detection. Judged by these standards, the results obtained were not encouraging. In only three out of the entire list, namely, numbers three, four and fourteen, were any effects ascertained, and these three proved so highly toxic to the rats when given in therapeutic amounts that it seemed questionable whether the effects on the tumors were attributable to the specific action of the drug, or to the general effect upon the health of the animal. At all events, none of the substances possessed that combination of properties which would make them available for the effective treatment of the rat tumor. The principal object of the investigation, therefore, failed to be accomplished.

I wish to acknowledge the constant help and advice of Professor Orndorff and of Mr. F. E. Carruth, without which this work would have been impossible.

## CONCLUSIONS

1. Living tumor cells are not penetrated by colloidal dyes.
2. The necrotic areas of tumors contain a larger amount of iodine than do the other tissues of the body after the intravenous injection of sodium iodide.
3. The necrotic areas of tumors present an intense discoloration after the intravenous or subcutaneous administration of dyes of the disazo group.

4. The discoloration of these tumor areas is very frequently associated with some discoloration of the liver, while the other tissues of the body remain macroscopically unstained.

5. The staining of the necrotic areas of tumors is not due solely to the death of the cells, inasmuch as areas of pulmonary caseation in the same rats do not present any discoloration.

6. The localization of colloidal dyes in necrotic tissues is not a simple physical phenomenon, subject to the laws of diffusion of fluids into non-living colloidal material. The diffusibility of the dyes through membranes, as also the electrical charge, the chemical reaction, and the chemical composition of both colloids influence the result.

7. A peculiar alteration in the color of dyes of the benzidine group occurs in necrotic areas. This has been described as metachromasia.

8. A series of new synthetic compounds analogous to congo red were injected into tumor bearing rats. No definite therapeutic effect could be determined.

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# PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

## EIGHTH ANNUAL MEETING

*Held in St. Louis, April 1, 1915*

### 1. Primary Spontaneous Tumors of the Liver in Mice

Miss Maud Slye of Chicago, Miss Harriet F. Holmes of Chicago (by invitation) and Dr. H. G. Wells of Chicago.

SUMMARY

Primary tumors of the liver are rare in mice, only twenty-three having been found in 9000 mice of all ages, dying natural deaths and carefully autopsied. All were liver cell adenomas, one showing malignant structure with multiple metastases in the lung, two malignant structure without metastases, and the remainder exhibiting all gradations from probable malignancy to simple adenomas. These growths were not encapsulated. No tumors of bile duct type, and no haemangiomas, were found. Sarcoma-like growths were discovered in four, but these were probably granulomas. There were three carcinomas secondary to mammary gland tumors, and one secondary sarcoma. None of the tumors was associated with cestode infection of the liver or other evident cause. All occurred in mice of highly cancerous ancestry, and none was found in approximately 3000 autopsies of mice of non-cancerous ancestry. The co-existence of liver tumors with other primary growths is high, there having been multiple tumors in nine of the twenty-three cases; in three both primary tumors were hepatic; in the other six, different organs were involved. Only four secondary tumors were found in the liver, three being metastatic from mammary gland carcinomas, and one from a mesenteric sarcoma.

DISCUSSION

*Dr. Ewing* (of New York): It is interesting to note the close resemblance between these hepatic tumors in mice and similar tumors in the human subject. Although Eggel found cirrhosis in about 85 per cent of reported hepatomas and in 50 per cent of biliary tumors, these high proportions are not maintained in other series of cases. Cirrhosis does not seem to be a necessary factor in the development of the typical hepatoma. It is more characteristic of the group of multiple tumors commonly called carcinomatous cirrhosis. In our laboratory we have five cases of typical hepatoma and in none was there cirrhosis.

*Dr. LeCount* (of Chicago): Is bile pigment present in the tumors?

*Dr. Richard Weil* (of New York): I should like to ask Dr. Wells to show what proportion of these twenty-three animals had a marked family history of tumor formation, or whether these tumors occurred in animals in which there had been no inbreeding. I think in a previous paper they have pointed out that the tendency to the occurrence of inflammatory and benign growths in the lungs in these animals with a marked family history took the form of malignant processes. I imagine that mild inflammatory conditions and small adenomata of the liver, such as we often see in the human being, might easily escape one's observation in autopsies on mice and rats, and I should like to know whether it would be possible to interpret these liver conditions that they describe here in harmony with the conditions they describe in the lungs, as simply an increased reaction in animals with an inherited tendency toward the formation of tumors.

*Dr. H. G. Wells* (closing): In regard to the relation of cirrhosis, my own experience has not been exactly that of Dr. Ewing. I have autopsied four cases of primary carcinoma of the liver myself. In all of them, there was a marked cirrhosis. Of course, the statistics are pretty definite on that point. There is one thing to be said about the carcinomata of the human liver: they have a peculiar incidence in the young. Of tumors growing in young people, a relatively large proportion are primary carcinomata of the liver. While in Vienna for two days, I saw three primary carcinomas of the liver autopsied, one of which was in a child of fourteen. That, however, has not been found in mice, namely that they occur at unduly early ages.

As to the question of bile production, we can merely state that there was neither macroscopical nor microscopical accumulation of bile pigment in these livers. I should have stated in presenting the material that none of these mice showed icterus and none showed ascites.

The question of inheritance I shall leave to Miss Slye to discuss.

*Miss Maud Slye* (closing): The question asked by Dr. Weil will be answered at some length in the paper which I shall read this morning

### 2. Renal Tumors of the Rabbit

Dr. E. T. Bell, of Minneapolis (by invitation), and Dr. A. T. Henrici, of Minneapolis (by invitation):

Only one renal tumor in the rabbit has been recorded so far in the literature. Report of two additional cases. Both these neoplasms resembled the embryonic kidney, being composed of masses of nephrogenous tissue showing various stages of differentiation into tubules; one contained a large number of well developed malpighian corpuscles. The tumors possessed the structure of the adeno-sarcoma found in the human kidney. Attempts to transplant them into other rabbits were unsuccessful.

### 3. The Tumors of the Japanese Waltzing Mouse and of Its Hybrids

*Dr. E. E. Tyzzer* (of Boston): A spontaneous carcinoma occurring in the Japanese waltzing mouse could be successfully implanted into over 95 per cent of the same race. It does not "take" when inoculated into the common mouse. If these two races be mated, the first generation hybrids "take" the inoculation in about 95 per cent. If hybrids be mated, the second and third generations of hybrids give practically 100 per cent of negative inoculations. Metastasis formation in mice growing this tumor occurs in a high percentage of cases. Massage of the tumor increases the incidence of metastases, which also occur earlier. This indicates the danger of undue manipulation of tumors. Filtration, centrifugation and drying of this tumor have each failed to indicate a causative agent apart from the living tumor cell.

A second tumor, originating in a Japanese waltzing mouse and apparently of the nature of a sarcoma, has been propagated for nine transfers during which it has grown in each of the 116 mice inoculated. This shows greater variation in its rate of growth than the preceding tumor; in some it remains about stationary for a long time while in others there is more rapid growth.

Partial retrogression has been observed during severe infection, but in no instance has the tumor entirely disappeared. No metastasis has occurred until recently, when secondary nodules have been found in the axillary lymph nodes and in the lung. The intravenous injection of this tumor and its resulting growth in the lung has not served to immunize the mice against the same tumor subsequently implanted beneath the skin. Neither does it immunize the mouse to the growth of the carcinoma previously described. Blood or serum of a rabbit immunized to this tumor is without curative action, even when repeatedly injected into the tumor itself.

### 4. Incidence and Inheritability of Spontaneous Cancers in Mice: Inheritability of Tumors of Specific Organs

*Miss Maud Slye:* The present study is based on stock which has now yielded over 10,000 necropsies, and has produced 722 cases of unquestioned tumor, many of these cases showing multiple primary tumors of different organs; the total number of cancers is thus considerably over 1000. Yet of this number there were only three tumors of the spleen, nine of the kidney, one to seven each of the stomach, rectum, face and jaw, adrenal, mesentery, chest wall, etc. The conclusion seems warranted that the provocative agent of cancer is of the type that stimulates rather than of that which destroys. Tumors of the ovary occur in unmated females about as frequently as in the mated. Is it not possible that the overproduction of unused egg cells may as readily be a stimulating cause for cancer in mice of high cancer ancestry as a specific lesion due to the production of young or to any other cause? Ninety per cent of the testicular tumors in this stock have

occurred in males long unmated, or never mated until after the appearance of the tumor. It seems likely that the over-production of unused spermatozoa may as readily be the provocation for tumor in mice of high cancer ancestry as a specific lesion in this organ due to bacteria, wounds, or other causes. Mammary neoplasms are about as common in unmated females as in the mated, and in this series they occur at a rather earlier age. It seems probable that the non-use of an organ ready for normal function may as certainly prove the irritating provocation for cancer as forced breeding and suckling in mated females of high cancer ancestry.

Whatever the nature of cancer may ultimately prove to be, this fact is certain: It follows the laws of heredity, not only in the transmission of cancer in general, but also in the transmission of cancers of specific organs, with an inevitableness which makes it a character that can be manipulated. There is transmitted a tendency for cancer to occur from a given provocation, such probably, as acute or chronic over-irritation, and, according to these observations, this agent is quite as likely to be of the constructive as of the destructive kind. The elimination, as far as possible, of all forms of over-irritation to the tissues of an individual of high cancer ancestry should go far to remove the provocation of cancer; and the eugenic control of matings, so that cancer shall at least not be present or potential in both sides of the hybrid cross, ought to eventuate in a considerable decrease in the frequency of human cancer.

DISCUSSION

*Dr. Richard Weil:* In spite of the fact that we have really only just begun the careful study of inheritance in tumors, the evidence at hand seems to point quite definitely toward certain important conclusions. Warthin, who collected a series of clinical histories, which are of great interest, from the material in the hospital at Ann Arbor, pointed out that there are, as far as his material has enabled him to judge, four inferences that can be drawn. In the first place, there is unquestionably a tendency to family inheritance of tumors. In the second place, there is a specific organ inheritance; that is to say, a tendency for these tumors to localize in certain parts of the body; and this tendency is transmitted. He pointed out that this holds true of tumors of the stomach, of the ovary and of the breast. Then, there is the tendency, as the family history is traced downward, to find these tumors occurring earlier and earlier in life. The first appearances of the tumor, as far as his histories showed, were in the decade between fifty and sixty; in the next generation, earlier still, in the decade between forty and fifty; and as one went farther on, if the family survived, still earlier. And the fourth conclusion that he pointed out was the tendency to tuberculosis, so extremely common in these families.

Now, these four inferences drawn by Warthin are in accord with those drawn by previous workers along the same lines, although Warthin's are very much more definite.

In connection with this question, I can report on one family history which bears out these views on inheritance. The patient was of the age of forty, a very well trained physician, a highly educated man, who presented himself with a large retroperitoneal tumor which impressed all those who have had the opportunity of examining him as a retroperitoneal sarcoma. His father had died of the same disease, which had been proved to be retroperitoneal sarcoma, at the age of forty-eight. This physician had four children; the first a son, living and healthy at the age of twenty; the second child, presented shortly after birth a glioma of one eye, which was removed—that child, a male, is still living; the third child, also a male, had glioma of both eyes and died; and the fourth child is a daughter, still living. The collateral family, most of whom have not reached the cancer age, have a marked tendency to tuberculosis, and there have been a large number of deaths in the family from this disease. So the history of that family has shown, first, a family inheritance; in the second place, a specificity of organ involvement; thirdly, a tendency for the tumor to occur earlier with each generation; and, lastly, a tendency to tuberculosis has manifested itself. Such tumors are fairly rare in type, and I think their occurrence in one family can be taken as indicating a certain relationship, and the presence of an organ specificity that will have to be worked out further. Then, there is the age of occurrence; in the first generation, almost a decade before the time of its appearance in the second, which was between the ages of forty and fifty; and in the third generation, in the first years of life.

There is one question upon which I should like to have Miss Slye's opinion, and that concerns the significance of these facts for protection. Levin, of New York, on the basis of his statistical studies, came to the conclusion that the protection against cancer, or the tendency to overcome cancer, was inherited, and that the tendency to develop cancer was a recessive factor; while Warthin, from his investigations, reached a conclusion exactly opposite, namely, that the tendency to develop the disease is inherited. It seems to me that Miss Slye has showed the same thing, and certainly in the family I have just reported the tendency to develop cancer resembles a dominant factor in some respects. Of course, the application of Mendelian terms to cancer inheritance is open to criticism.

*Dr. Moyer S. Fleisher:* In regard to what Dr. Weil has just said, in Dr. Loeb's work on the inheritance of cancer in mice he has crossed two races of mice, one in which the rate of cancer was low and another in which it was high; he has also used races in which the rate was medium. He has not found, so far as his work has gone, a distinct regularity in his results; so that, at the present time, with the experience he has had, it does not seem definite whether the tendency to develop cancer is recessive or dominant. Certainly other factors, probably many other factors, must be taken into account.

*Dr. James Ewing:* Miss Slye has undoubtedly shown to the satisfaction of this Association that the inbreeding of mice may intensify an hereditary tendency to cancer. Under these conditions heredity proves to be a very important etiological factor. The elaborate plan and great detail with which these studies have been conducted make these experimental results of great value, but the extent to which they may be applied to the various forms of human tumors seems to me a somewhat different problem. I am one of those who believe that the influence of heredity in human cancer can be safely determined only by actual observation on the human subject. I further believe that the facts must be separately determined for each particular form of cancer, since many forms of cancer are quite different diseases. We already know that there is a pronounced family tendency to glioma of the retina. There are also remarkable instances of the occurrence of several cases of cancer of the stomach in the same family. Yet in the great majority of cases of human cancer it is impossible to trace any hereditary influence, so that exceptions to this rule at once become very notable. In cancer of the tongue very extensive clinical observation has shown that this disease almost invariably arises as a result of syphilis, bad teeth, or the use of tobacco, and that any hereditary element may be ignored.

After an hereditary influence has been demonstrated to affect the occurrence of a given form of cancer, I think it is unwise to assume that such an element exists in, or that any general rule applies to, all forms of the disease. This matter has an important practical bearing. It would be highly unfortunate if the public were instructed that cancer as a whole is an inherited disease, especially if it were not so. Such a doctrine would greatly increase the difficulties of acquainting the public with many facts of much practical importance, some of which may enable the layman to escape certain forms of cancer. A campaign of education is now being conducted by the American Society for the Control of Cancer, and the difficulties of conducting this campaign would be increased if the doctrine of the hereditary origin of human cancer were extensively spread. If this hypothesis be correct, then it must be spread, but not before it is fully demonstrated. It would be especially unfortunate if it should be made a propaganda by the daily press before it has stood the test of medical criticism. Until such a test has been passed, I think one should be very conservative.

*Dr. E. E. Tyzzer:* I should like to say just a word. I cannot fully agree with Dr. Ewing in these statements. I have insisted for a long time on the point exemplified by Dr. Weil's statements, for it appears to me that certain activities supposed to be directed towards the control of cancer will probably result in a further increase of cancer unless the importance of the work in genetics is recognized. The human cases cured before the end of the reproductive period will tend to increase the number of individuals with a predisposition to cancer in the next generation. It seems to me that it is with investigation

in genetics—that is, from the standpoint of the actual elimination of cancer—that the hope of the future lies. If to-morrow we had a cure for a large proportion of cancer cases, the next generation would probably see a far greater increase in the incidence of cancer than is asserted to be taking place at present.

*Miss Slye* (closing): Regarding the question whether cancer is a recessive or a dominant, it must be confessed that there has been for a number of years a great deal of difficulty with these factors in problems quite outside that of cancer, and the question is not always easy of settlement. I do not happen to have with me to-day the data that would demonstrate perfectly my clearest tenets in this field. Those charts were presented a year ago before this society. In those charts, for example, in Strain 413, where neither parent was cancerous, but where the maternal grandmother had cancer, cancer comes out in the second filial generation. This is just what one would expect it to do if cancer were a recessive, as, in fact, I believe it to be. All of my results are best explained on that basis. If you will allow me to select what I can from that which is here, I shall be able to make this point clear. (Illustrated by charts and blackboard demonstration.)

Consider the well-known Mendelian diagram. By crossing a common house mouse with an albino, a first filial generation of greys is obtained. These greys, if bred out, will give three different lines: one straight house-mice, one straight albino, and one heterozygous. The heterozygous lines, if inbred, will continue to split until the end of time, giving the same three types.

Now, the rôle of the heterozygote in this whole question of cancer, as in every other problem of heredity, is, in my judgment, extremely important. It is possible if one continues generation after generation to mate a heterozygote with a dominant, never to produce any albinos, or never to produce any cancer, as the case may be; although in the one case albinism, and in the other case cancer, is potential in such crosses until the end of time. But if such a heterozygote be mated with another heterozygote or with a recessive, the recessive character comes out in the first filial generation, be it albinism or cancer. The cancer charts which I presented before this society a year ago demonstrated this completely. I charted this uterine tumor case largely because it did show exactly that. In the second filial generation, every individual whose case is known had uterine tumor, where both grandmothers had uterine tumors and neither immediate parent had. In every case where cancer has come out in the first filial generation in one of my strains, it has been by the union of two heterozygotes, or by the union of a cancerous individual with a heterozygote.

My answer to Dr. Ewing's remarks on inbreeding in its relation to the cancer problem is this: Inbreeding, as such, has nothing to do with the production of cancer. My strains of non-tumorous mice have been inbred in just the same way as the tumorous, but no cancer has occurred in them, even where inbreeding has been continued for twenty

to twenty-eight generations. The method of the laboratory in deriving fixed strains must always be to breed out (that is, to "inbreed") the progeny from a hybrid cross. Only so can we definitely know what was derived from any hybridization. But in the transmission of albinism, an albino from Strain 139 mated with an albino from an unrelated series, Strain 94, will just as certainly produce nothing but albinos as when breeding in its own strain. And the mating of a heterozygote from such a cross with a wholly unrelated heterozygote from another cross with cancer behind it, will just as inevitably produce cancer in the immediate offspring as the mating of two heterozygotes from the same litter.

Just so, as shown in every hybrid chart presented before this society last year, the mating of cancer-bearing individuals derived from wholly different strains will effect exactly the same transmission of cancer to the progeny as does inbreeding within cancer strains.

In the general matter of the increase of cancer, outbreeding will do more to increase cancer than inbreeding ever could; for inbreeding (mercifully, where an undesirable character is concerned) has one quality which outbreeding lacks; viz., it kills off the strain. Note Chart 1, Strain 139; this strain, carried through many generations of inbreeding, was dying out, when the introduction of a member of it (Female No. 529, who had had no young by her brother, Male No. 553) into a hybrid cross with Male No. 242 from an unrelated strain produced a prolific and highly cancerous family, Strain 146, and initiated a whole line of cancerous individuals.

Again, the mother (No. 293) of this female, when mated with her brother produced but a few offspring that lived to maturity, though when cross-bred with a wholly unrelated male she produced Strain 65 with its offshoots, a persistent and highly cancerous strain which introduces cancer into any strain with which it is hybridized.

In conclusion, I should like to express my thorough agreement with what has just been said by Dr. Tyzzer. This is not a case where ignorance is bliss; rather it is one where ignorance is a crime against the race. It seems to me, Dr. Ewing, that if we could educate the public to the certain knowledge that when we deal with heredity we are dealing with something just as inevitable in its results as the reaction of chemicals in a test tube, then and then only may we hope to stop some of this intricate hybridization of cancer-bearing individuals which inevitably increases human cancer, and which, if persisted in sufficiently long, will eventuate in strains no one of which will be wholly free from cancer.

### 5. The Immunological Relations of the Rous Chicken Sarcoma

*Dr. William H. Woglom* (of New York): Dr. Woglom stated that he had been unable to induce any immunity to the Rous chicken sarcoma by the method usually effective against tumors of mice and rats, that is, by previous treatment with embryos of their own race.

### 6. Transplantation of a Fowl Sarcoma (Rous) By Injection of Filtered Citrated Blood Plasma from an Infected Fowl

*Drs. G. H. A. Clowes and B. T. Simpson* (of Buffalo): Blood was drawn from the vessels of the neck in two chickens carrying this spindle-cell sarcoma, and was immediately introduced into cold aqueous citrate solution. The plasma was separated by centrifugalization, and one portion injected without further treatment. A second was filtered through a coarse grade Berkefeld bougie, tested subsequently and found to be impervious to cultures of Bacillus prodigiosus. This portion, like the former, was introduced, into the breast muscle in a number of chickens. Tumors were produced by both the filtered and the unfiltered portions. In view of the fact that the injection of whole blood, or of corpuscles alone, or of serum from defibrinated blood have all hitherto given negative results, the conclusion is tentatively drawn that the addition of a small amount of citrate to the blood serves as an important element.

### 7. Chemical Studies on the Serum of Fowls Bearing the Rous Sarcoma

*Dr. Casimir Funk* (of New York): Within recent years several methods for the serum diagnosis of cancer have been advised, but all have been found unreliable. In contrast to these procedures, which are based on the biologic properties of the serum, the present paper deals with certain chemical differences. As a preliminary study, the serum of chickens inoculated with the Rous sarcoma was analyzed; the average figures obtained from twenty-two malignant cases, when compared with twenty-two normal serums, showed a diminution in the total nitrogen, phosphorus, sulphur, and chlorids, but an increase in ammonia nitrogen and sugar. The differences, which were apparently due to anemia, were so pronounced because of the short period which fowls live after inoculation with the tumor. Analogous results were obtained in a few instances with rat serum from animals bearing a sarcoma. In human serum, the chemical analysis has failed thus far to reveal any important chances, so that a more detailed analysis and investigation of the serum will be necessary.

### 8. Studies on a Cell (Zehbe's) Found in Cases of Human Carcinoma and Mixed Tumor

*Dr. Georgine Luden* (of Rochester, Minn.): During the routine examination of parathyroids, attention was attracted by a peculiar pathologic cell, found repeatedly in cases of carcinoma which had come to necropsy. This cell belongs to a group known among pathologists as endothelioid, epithelioid, or plasma cells. Zehbe has described it very minutely, though his description contains three errors: the extremely

small nucleus to which he refers is the nucleolus, his foamy protoplasm is the nucleus itself, and his numerous mitoses are particles of nucleolar chromatin. In three cases of carcinoma this cell served as "indicator," revealing carcinoma in organs in which it was not suspected; and in a case of hemachromatosis, supposed to be of tuberculous origin, it led to the discovery of generalized malignant disease. In each of these cases the pathologic findings were not of a metastatic nature, but showed malignant degeneration in histologically different tissue. Downey has pronounced the cell a pathologic variety of fibroblast. In every instance its presence is accompanied by three prominent changes: increase of connective tissue, malignant proliferation of very diverse types of cells, and a chemical affinity for hematoxylin and other basic dyes in the parenchyma in the immediate vicinity of these cells and, in more advanced stages of malignancy, in the cells themselves. In consideration of these facts and the chemical reactions which this seems to imply, the assumption does not seem to be unwarranted that three factors are needed for the effective progress of malignancy: (1) a disturbed internal chemistry; (2) an outside irritant, and (3) a local chemical defenselessness of the tissue which is first to succumb.

The following conclusions may be drawn: In the organs of patients dead of carcinoma there may be found a peculiar cell (probably a pathologic type of fibroblast) which occurs in the presence of primary foci or of metastases. The association of this pathologic type of fibroblast with malignant changes is so constant that its presence serves as an indicator of the presence of malignant changes. The cell may be known by its oval nucleus 5 by 10 microns in diameter, sometimes by its faint zone of cytoplasm. The development of this pathologic connective tissue cell in association with carcinoma, suggests the presence of a widely distributed chemical factor fundamental in the production of cancer.

### 9. Comparison of the Immunizing Effects of Subcutaneous and Intraperitoneal Administration of Tumor Cells Against the Growth of Carcinoma in Mice

*Dr. Major G. Seelig and Dr. Moyer S. Fleisher* (of St. Louis): Both intraperitoneal and subcutaneous tumors have a mutual inhibiting action on each other, provided they are virulent or moderately virulent. An intraperitoneal tumor, however, has relatively a very much stronger inhibiting power than has a subcutaneous growth. The number of "takes" after intraperitoneal inoculation is smaller than after subcutaneous, and the rate of growth is less rapid. The intraperitoneal injection of unheated, as well as of heated tumors, yields a number of "takes" approximately 40 per cent less than does subcutaneous inoculation. Notwithstanding the lower percentage of "takes," the immunizing effect of intraperitoneal growths is markedly greater than that of subcutaneous tumors. A subcutaneous neoplasm with markedly

diminished virulence may exert a favorable influence on a second subcutaneous tumor of diminished virulence. In the same way, a subcutaneous and intraperitoneal tumor may, under certain conditions, mutually exert a favorable influence on each other.

### 10. The Rôle of Inflammation in the Immunity of Mice to Implanted Tumors

*Dr. E. E. Tyzzer* (of Boston): The common mouse is found to be invariably refractory to the growth of implants of carcinoma derived from the Japanese waltzing mouse. Such implants, however, grow for a period of at least six days, during which they do not excite any marked inflammatory reaction. Following this period, there occurs an inflammatory reaction which is accompanied by degeneration of the tumor and eventually by its disappearance. A comparative study was made of the tissue reaction around implants of this growth into naturally immune common mice and into common mice which had previously been inoculated with the tumor. It was found that the inflammatory reaction was initiated at least three days earlier in the latter group, and that destruction of the tumor graft also occurred much more rapidly. An investigation of the reaction of mice to a tumor of their own race in normal, as compared with immunized individuals, showed differences similar, although less pronounced.

The following hypotheses are the more important among those that have been offered to explain immunity to inoculated tumors.

1. Antibody immunity acting through cytolysins for tumor cells. There is little evidence to indicate that tumor cells undergo cytolysis in immune animals.

2. Inhibition of connective tissue proliferation through the production of some agent which renders the tumor implant unable to provide itself with a stroma. Injury and inflammation in the tissues surrounding the implant are not recognized as important by the supporters of this hypothesis.

3. Immunity to microörganisms which protects against the subsequent growth of implanted tumor.

4. Sensitization. The production of material in the immunized animal which, on combining with the products of living tumor cells, causes an injury that is most marked in the tissues about the implant. The destruction of the tumor is secondary to this, and results through the isolation of its cells from the healthy growing tissues necessary for its nutrition. While the last hypothesis appears to be supported by the data at hand, as well as by much of the evidence furnished by others, the possibility of applying the other hypothesis to certain limited groups is not excluded thereby.

The following conclusions may be drawn from the results of this investigation:

Repeated inoculations of the various tumors employed do not produce sufficient immunity to overcome an established tumor resulting from the first implant.

It has been thus far impossible to immunize the Japanese waltzing mouse against tumor "J. W. A.," not only by repeated injections of large doses of dried tumor in suspension, but also by treatment with defibrinated blood or living tumor. The individuals of this variety of mouse show such uniform susceptibility that transference of the tumor from one individual to another appears not to involve any appreciable modification of its environment. The lack of immunity reaction is seen in the frequency of metastases and in the absence of retrogression. This tumor, as propagated in the waltzing mouse, reproduces very closely, therefore, the conditions under which spontaneous tumors develop.

### 11. Transplantation of Tumors Into Foreign Species

*Dr. Casimir Funk* (of New York): The following experiments were performed to test the validity of Ehrlich's athreptic hypothesis, which would explain the failure of tumors to grow in foreign species by the assumption that they lack their specific food supply. Two series of rats, one of which had been fed with the tumor for some time previous to inoculation, were engrafted with a mouse chondroma. In the control rats the mouse tumor failed to proliferate, but in those fed with the chondroma several tumors identical with the growth inoculated were obtained. One of these was transplanted back into mice, and again into prepared rats. The chrondroma continued to grow in rats for three generations (more than seven weeks).

#### DISCUSSION

*Dr. James Ewing:* I should like to ask how often Dr. Funk repeated this experiment.

*Dr. Casimir Funk* (closing): The experiment was repeated twice. In the controls which were not fed with tumor, growths did appear though they were smaller and fewer in number.

### 12. The Action of Radium on Transplanted Tumors of Animals

Dr. F. C. Wood, of New York, and Dr. Frederick Prime, Jr., of New York (by invitation).

Dr. Wood spoke of the necessity for applying quantitative methods to investigations of the effect of radium upon cancer. Most of the reported clinical observations were of little value; thus, in some cases the tumor had never been microscopically diagnosticated, or the length of exposure and the distance of the radium from the tumor were not given, while even the amount of radium employed could rarely be estimated. An extensive series of experiments on mouse and rat tumors had shown that very much longer exposures than had hitherto been

regarded as necessary were required to kill cancer cells. The results, plotted in a curve, showed that when the distance from the tube was the same the only other factors involved were the time of exposure and the quality of radium element employed. Dr. Wood said that the exposure demanded by the outcome of these experiments approximated that requisite in the case of human carcinoma.

DISCUSSION

*Dr. Georgine Luden:* I should like to ask Dr. Wood whether he noticed any change in connective tissue reaction.

*Dr. Wood:* I do not believe that the connective tissue changes are primary; in most cases they follow the destruction of the cells of the carcinoma. While one is waiting for fibrosis to occur the carcinoma cells may metastasize at a distance; one can not, therefore, depend upon fibrosis for a cure, but must attempt to kill all the malignant cells immediately.

*Dr. Ewing:* Is it not possible to consider still another factor, namely temperature, as influencing the effect of radium? Several observers have found that increased temperatures augment the effect of the X-ray, and since the activity of radium is very similar the same relations to temperature probably hold true.

*Dr. Wood:* I do not think that the temperature has any effect. The tumors were exposed in vitro, in the incubator, at room temperature, and in the cold, and no variation was noted. The reason for treating in the cold was, of course, to preserve the viability of the tumors so that they could be inoculated at the end of twenty-four to forty-eight hours.

I think the vascularity of a tumor has a great deal to do with its rapid shrinkage under radium. There is no question but that thrombosis of the vessels is easily produced in glandular carcinomata and sarcomata, and a large portion of the tumor may slough out; but this is only a temporary effect, since there remains a shell of healthy cells from which recurrence takes place. This peripheral zone is nourished by well formed blood vessels from the capsule of the tumor and can not be destroyed by radium to any extent. In the firmer, less vascular tumors, such as epitheliomata, the thrombotic effect is not noticeable, and there is no sudden change observable in the tumor, but only a gradual shrinkage after the death of the cells has occurred.

*Dr. E. E. Tyzzer:* Dr. Duane and I have been working at intervals on the effects upon mouse tumors of radium and its products, more especially, however, of its products. The first procedure undertaken was the injection of *radio-active salt solution* into the veins of animals bearing tumors. This had very little destructive effect upon the neoplasm, although there was some retardation of growth; but we lost most

of the animals. Then tubes containing *emanation* were placed in the tumor. This caused considerable destruction, and in a number of instances seemed to destroy the entire neoplasm; but in all these cases also, as well as in some where destruction was not complete, the animal succumbed. The third mode of attack was to insert into the tumor tubes containing *deposited activity*. In this way it is possible to deal the malignant tissue a very short, severe blow, for the deposited activity has a life of only a few hours. Although deposited activity is effective for only a short time, large amounts may be employed. The method, however, has not as yet been followed by any very favorable results.

In regard to the stimulation of neoplasms, Dr. Kellert, who exposed a number of tumor-bearing animals to X-rays, found by transplanting the exposed tumor that growth of the implants was tremendously stimulated. He thus observed an effect similar to that which has been described by Dr. Wood.

As for Dr. Wood's conclusion regarding the principle involved in radium treatment, which is that an actual killing of the cells by a physical agent is the only process that takes place, it seems to me that this is not borne out by all the results obtained up to the present time. We do not know just how the changes take place following exposure to radium; we do not know how they are brought about, for example in leukemia, where exposure of a very small area of the spleen will produce the most profound results. Of course, such spleens may not be regarded by all as malignant tumors, but it is living tissue which is affected nevertheless, and the results are very profound.

*Dr. Richard Weil:* I should like to ask Dr. Wood whether his observations throw any light on Wassermann's attempted analysis of the action of radium on new growths. Wassermann attempted to differentiate between the effect on nutrition and the effect on generation, and he invented a terminology of "ceptors," which probably has no strict biological application. Yet it has always seemed to me that the theory might contain a kernel of truth, for anything which would interfere with nutrition would eventually interfere with generation.

*Dr. Wood:* I have felt that Wassermann's paper was quite unimportant in connection with radium work. Most of it is devoted to discussing the question of "nutriceptors" and "genoceptors," about which we know nothing, and I do not believe that the methylene blue reaction is a sufficient test for determining the viability of the cell. There is no question that the radiation of lower animals (protozoa, eggs of Ascaris, etc.) has resulted in death after a number of days, but these hypothetical "nutriceptors" and "genoceptors" certainly do not explain anything. What causes cell death following exposure to radium we do not know.

The practical aspect of the work which I have just reported seems to me to lie in the attempt to determine the effects of radium on a

standard tumor. We know how greatly cells vary in their power to resist this substance; that the lymphocytic tumors, for example, are often extremely susceptible, while other types are very resistant. The mouse carcinoma is a more or less standard variety of glandular tumor which does not differ much from the human type. It is important that we determine the lethal dose of radium, because unless we know this accurately the danger of undertreating, and thereby stimulating, the growth, is always present.

### 13. The Treatment of Parotid Tumors by Radium

*Dr. Richard Weil* (of New York): Dr. Weil reported a case of parotid tumor which had been successfully treated with radium in the Memorial Hospital of New York. A number of other favorable results with tumors of the parotid have been reported, but in no instance has the microscopic character of the tumor been described. In the present case, a small piece removed for examination was diagnosed by Dr. Ewing as adenoid cystic epithelioma. The appearance corresponds to that frequently described as cylindroma. The sections show cells resembling epithelium, and arranged in cords which have extensively infiltrated and destroyed the muscle bundles. No tissues characteristic of the "mixed tumors" were discoverable. The patient was a female aged thirty-eight. The tumor, which had been slowly growing for seven years, was seated in the parotid region and involved the entire lower half of the ear, and of the upper part of the neck behind the ear. It was firmly adherent to the deeper structures and in part to the skin. There was complete facial paralysis on the right side. The radium was inserted into the tumor through an incision, eighteen such applications being made. At the end of six weeks the tumor had practically disappeared. Up to the present time, after an interval of more than one year, there has been no evidence of recurrence. The facial paralysis, however, has not been benefited.

#### Discussion

*Dr. F. C. Wood:* I have long been interested in this group of tumors and have seen, probably, some one hundred and twenty of them. Some years ago I examined all the specimens preserved in the laboratory of the College of Physicians and Surgeons, New York, and found that only about fifty per cent of these had recurred, even with the imperfect operative procedures which were then employed, most of the tumors dating back twenty or thirty years.

I think that almost all these tumors might be successfully removed, were it not for the difficulty of operating so as to avoid cutting the facial nerve. This tumor is of a type which is relatively harmless. I have followed up a number of them of similar morphology, observed in St. Luke's Hospital during the last few years, and have found no recurrence. I have seen other cases, however, which did very badly under radium

treatment. The carcinomata, squamous cell epitheliomata, and sarcomata which occasionally arise in salivary gland tumors after they have existed for a good many years, are of an entirely different type, extremely malignant, and not at all susceptible to radium therapy. This case of Dr. Weil's is certainly very interesting, and illustrates the necessity for careful microscopical study of tumors before the application of radium. Growths containing cartilage are, as a rule, resistant to radium, though in general no more malignant than the basal cell epitheliomata of the face, which, as we all know, can often be treated more efficiently with radium than in any other way.

*Dr. Richard Weil* (closing): I should like to say that the tumor reported was one which cannot be accurately described as malignant, in spite of certain histological features. It invaded and infiltrated the neighboring tissues, destroyed a large part of the ear, was densely adherent, and had apparently infiltrated and destroyed the facial nerve. Yet, on the other hand, it was slowly growing and did yield to radium.

### 14. Chemotherapeutic Experiments on Tumors

*Dr. Richard Weil* (of New York): The paper contained a detailed discussion of certain special problems in chemotherapy. It was shown that dyes of the benzidine group are not taken up by the living tumor cells of mice or rats. The necrotic portions of these tumors, however, take up the dyes of this group with considerable avidity. After intravenous injection, the necrotic parts of the tumor appear to be stained more intensely than any other tissue, except, perhaps, the liver. The process of staining is not one of simple diffusion, inasmuch as these dyes are colloidal. It is possible that the relatively intense staining of these areas is simply due to a defective vascular supply which results in the less perfect removal of the dye. Many of the dyes undergo a change of color in the necrotic areas, which is similar to the metachromasia seen in staining amyloid. Similar color changes could be produced in the test tube by adding solutions of the amino acids to the dyes. Compounds of certain dyes, notably Congo red, with copper, selenium, mercury, and formalin, were injected intravenously into rats carrying tumors, but no therapeutic effects were observed.

#### DISCUSSION

*Dr. H. G. Wells:* I am naturally very much interested in this work. As regards the fact that some of the colloidal dyes diffuse into the tissues in a way that the larger colloidal molecules of proteins have been found not to do, I, of course, have already learned that from Dr. DeWitt's experiments. But there is one thing I do wish to speak of, and that is that in all this vital staining work with dyes obtained from the German manufacturers it has been found, apparently by every one working with these dyes, that they are absolutely unreliable as regards the

labels and the contents of the bottles. Dr. Paul Lewis, also, has been obliged to prepare his own dyes, as we have had to do. The label and the color of the dye will agree, it is true, but the chemical composition may be entirely different in dyes of similar color bearing the same label.

*Dr. Richard Weil:* I have had the same experience with German dyes, but have been able to get better preparations by communicating with the New York managers of these companies, who have sent to Germany for special samples. In some cases, however, I have not relied even on these, but have used chemically pure dyes prepared by Mr. Carruth.

# TUMOR IMMUNITY

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In a consideration of tumor immunity it appears desirable to discuss resistance to spontaneous tumors and to implanted tumor separately. Although results obtained with experimentally implanted tumors have contributed to the biology of tumors, these results can not be applied directly to spontaneous tumors and this is especially true with respect to immunity. It has long been recognized that immunity to implanted tumor gives no assurance against the subsequent development of spontaneous tumors. It is quite impossible to present within reasonable space a comprehensive review of all investigation in tumor immunity, so that in the attempt to furnish an adequate explanation of the phenomena relating to tumor immunity, such data as appear to bear directly on the subject will be discussed and correlated as far as possible.

## RESISTANCE TO SPONTANEOUS TUMORS

The individual who is without any form of tumor may in a certain sense be regarded as immune, and the mechanism for regulating the growth of tissue as one of immunity. The normal individual may, however, develop a tumor at any time and, with the limitation of active growth to a relatively small group of cells, it appears improbable that there is any abnormality of the growth regulating mechanism but rather a local derangement of the group of tissue cells from which the tumor arises. This view is supported in that Haaland has observed that tumors may be transplanted to a normal animal as readily as to one in which a tumor has developed and also in that it is found that young healthy animals are more favorable to the growth of implanted

tumor than older ones. Since natural resistance, once a malignant tumor has become established, appears to be usually ineffective, many attempts have been made to increase it through various artificial procedures.

*Spontaneous regression.* Recovery from a tumor which has shown malignant characteristics is rare. A number of such recoveries have, however, been recorded and incomplete excision has in a few rare instances been followed by complete cure. That there is also spontaneous recovery from certain border line conditions appears probable. Evidence of local disappearance of tumor is more frequently met with—for example a scirrhous carcinoma of the breast may disappear in certain regions while actively growing in others.

*Reaction of tissues to tumor.* The histological study of tumors also discloses reactions of the surrounding tissue, some of which are unquestionably favorable, others distinctly unfavorable to the growth of the tumor tissue. Certain uterine carcinomata, for example, excite a pronounced infiltration of eosinophiles and in such cases the reactionary tissues may exceed in amount the essential tissue of the tumor.[1] Certain carcinomata of the lip, as well as the border line conditions in this region, show marked inflammation in the tissue near the abnormal epithelium and degenerative changes in the contiguous connective tissue are frequently prominent. In other instances proliferative changes in the supporting tissues are most marked. This is seen in adenomata of the breast and in the papillary tumors of the ovary. The proliferation may be present in such a degree as to give the appearance of mixed tumor or of sarcoma. Such a degree of connective tissue proliferation has attended the transplantation of certain carcinomata in mice as to constitute at first a mixed tumor and later on, after the elimination of the epithelial elements, a sarcoma. Thus Ehrlich, Loeb, and Haaland have each produced sarcomata experimentally by the inoculation of epithelial tumors. With certain carcinomata a dense scar-like tissue is formed which tends to bring about the atrophy and dis-

[1] Noted in a case from the collection of the Department of Pathology, Harvard Medical School; also noted by Mallory.

appearance of the tumor epithelium through pressure and interference with nutrition. Accordingly it is possible to distinguish a great variety of reactions on the part of the supporting tissue in response to the influence of tumor cells. Some are distinctly antagonistic, others indifferent, others favorable to the growth of the tumor. It is obvious that unless the neoplastic tissue is of such nature as to stimulate a reaction in some degree favorable to its support and nutrition, its continued growth will be impossible. Such reactions furnish definite evidence of the biological variation of the tumor from the somatic tissue from which it arises, a subject which will be further discussed later on.

*Relation of resistance to metastasis.* In the distribution of metastases there is considerable evidence of organal immunity. Certain organs may be free from secondary tumors in cases in which there is every indication of a general dissemination of the tumor cells, and emboli of tumor cells have been found undergoing degeneration. Certain tumors are widely distributed in certain tissues—for example, lymphomata which grow profusely throughout the lymphoid tissue—while other tissues are for the most part exempt from invasion. On the other hand, epidermoid carcinomata are observed which, while infiltrating the tissues diffusely, are at the same time being reduced to inert masses of cornified epithelium wherever their cells have lodged in the lymph-nodes. However, on the whole, it is impossible to ascertain in most cases whether the distribution of the tumor is determined by its biological peculiarities with respect to the invasion of vessels or by the unfavorable conditions furnished it by certain organs and tissues. Adequate vascularization is probably an essential factor in the nutrition of most tumor metastases. A more definite knowledge of the principles governing organ or tissue exemption from metastasis will be acquired when the experimental method is more generally applied, and animal tumors, since they are available in considerable numbers, are suitable for this problem.

The opinion has been expressed that metastasis is to some extent governed by the resistance of the individual. Sticker recognizes a pre-metastatic period in the growth of tumors during

which the resistance of the body is sufficient to destroy all cells which may enter the circulation. Gay, on finding that a certain proportion of rats bearing the Flexner-Jobling tumor recovered if reinoculated during this period, adopted Sticker's views in the explanation of his results. The author has found that by the forcible manipulation of a tumor of the Japanese waltzing mouse, metastases may be produced experimentally and it is not only possible in this way to reduce artificially the premetastatic period but also, by ascertaining the time required for the tumor emboli to become visible nodules, to show that the natural premetastatic period is actually considerably shorter than it had appeared. It has been repeatedly shown that animals, on developing tumors from transplants, frequently become more unfavorable to the growth of subsequent implants. Although it is not improbable that the presence of a local tumor may stimulate a reaction which is unfavorable for the development of tumor emboli, experimental evidence of this is lacking, and the peculiarities of the individual tumor with respect to its ability to disseminate its cells should be taken into consideration. Tumors which metastasized in the individuals in which they originated have a greater tendency than others to metastasize during propagation.

Certain points with respect to conditions governing the growth of tissues are readily determined in human beings. The following observations were made with respect to the persistance of transplants of the abnormal tissue in myelogenous leukaemia and Hodgkin's disease during the course of certain procedures that were undertaken by the members of the staff[2] of the Huntington Hospital with the view of testing the efficacy of autologous vaccination which was at that time on trial in the treatment of tumors.

*Myelogenous leukaemia*

*Case C. I. 12. 5.* This patient, a woman 37 years of age, showed marked enlargement of the spleen and a white count varying from 91,000 to 496,000 while under observation; duration at least two years.

June 3, 1912. A cubic centimeter of leucocytes collected from citrated blood was injected subcutaneously.

[2] Thomas Ordway Ellis, Kellert and the author.

June 5, 1912. Slight tenderness and induration at site of inoculation. This had entirely disappeared at the time of the next observation several days later.

Larger quantities of leucocytes were employed in subsequent injections—15 cubic centimeters being used in one instance—but these were always quickly absorbed.

June 29, 1912. One cubic centimeter of blood was drawn from a vein and injected immediately beneath the skin of the upper arm.

June 30, 1912. The blood had been almost wholly absorbed and there was no discoloration present.

*Hodgkin's disease*

*Case I. 12. 43.* A girl, seventeen years of age, with enlargement of the glands of the neck, axilla, and mediastinum; duration of disease, 4½ years.

March 10, 1912. A small nodule was excised for diagnosis. A small portion of this was immediately inoculated subcutaneously near the insertion of the deltoid muscle by means of a trochar. Following this the implant was palpable and the overlying skin slightly reddened.

March 13, 1912. No reddening and the implant had diminished in size.

March 19, 1912. No trace of the implant remained.

These observations show that the abnormal cells in these two diseases do not find the conditions furnished by the subcutaneous tissues favorable for their development.

*Diagnostic tests and curative sera.* A considerable portion of the work on immunity to spontaneous tumors has been done in connection with human cases and falls naturally into two divisions; first the reactions which might serve as diagnostic or prognostic tests, and second, measures which have been carried out with the view of effecting a cure by some general form of treatment. Among the proposed diagnostic methods may be mentioned Freund's reaction, the meiostagmin reaction, the hemolysis test, Abderhalden's test, the cobra venom test, and complement fixation. As possible curative agents trypsin, sera from resistant human cases, living tumor used as an autologous vaccine, tumor extracts and cell ferments, dried animal tumors, and the transfused blood of normal individuals have been employed. Since

the various tests each require separate consideration no discussion of them will be undertaken. It is notable, however, that up to the present time these have in general been found either unreliable or unpractical. The general failure of the long list of therapeutic measures for which claims have been made is also a matter of common knowledge.

## IMMUNITY TO TRANSPLANTED TUMOR TISSUE

The subject of immunity to transplanted tumor tissue has been extensively investigated. Although the transplanted tumors of rats and mice are especially favorable for experimentation, there has been more or less discrepancy in the results obtained by different investigators. Explanation for this is found both in differences in the biological character of the various tumors employed as well as in differences in the character of the animals with which these are tested. Russell has employed in his investigations a great number of tumors, the immunizing qualities of which he has determined by testing them upon mice of the same breed with adequate controls with respect to age and weight. The author has attacked the problem from the diametrically opposite side and has tested the reaction of different varieties of mice to a single tumor. Thus while in the experiments of one a stock of tame mice was taken as the indifferent or constant and the tumor as the variable factor, in the experiments of the other a single tumor served as the constant and different varieties of mice and their hybrids as the variables.

*General features.* Immunity to transplanted tumor has as its basis the reaction of the organism to a foreign cell. It has been shown that animals treated with normal cells may manifest an increased resistance to implanted tumor and also that animals treated with tumor tissue may subsequently show pronounced immunity to the same tumor or to other tumors. The condition of resistance to implantation of various types of tumors by the previous injection of a single tumor has been termed *pan-immunity* by Ehrlich. Differences in the immunizing qualities of various tumors has been definitely established by Russell, who has found that in general the poorly growing tumors serve as

the more "efficient antigens." Loeb, Haaland, and the author have each shown that tumors may be readily reimplanted in the individuals in which they arise: *autoplastic grafting.* When a tumor is transferred to other individuals of the same race a variable number of failures of growth usually occurs, and greater difficulty is encountered in the successful implantation of tumor tissue in other races and varieties: *homoioplastic grafting.* Practically invariable failure follows the implantation of tumor in other species: *heteroplastic grafting.* The degree of the immunity which develops thus depends apparently on the foreignness of the immunizing cell with respect to the organism into which it is introduced. The more foreign cells accordingly serve as the more effective and the more closely related cells as the less effective antigens.

*Antigen.* Immunity to tumor tissue is evidently excited by some subtle product of cell metabolism eliminated in small amount, but over considerable periods of time. Repeated attempts have been made to immunize with dead tumor or other tissue products, but notwithstanding several unconfirmed reports of success such procedures have in general proved unsuccessful. The great majority of those who have investigated the subject have thus found that the injection of living cells is essential in the production of immunity. While such treatment may serve to prevent the growth of subsequently implanted tumor, it does not bring about the retrogression of established tumors. The regression of implanted tumors of large size may, however, occur spontaneously, and the animals are then immune to further implantation. Tumors have been found which grow in practically 100 per cent on implantation but which subsequently all retrogress if left for a sufficiently long time.

*Histology of the tissue reactions to transplanted tumor.* When a tumor such as the carcinoma (J. w. A.) of the Japanese waltzing mouse is implanted into a series of common mice, i.e., into individuals of an alien race, its continued growth is invariably prevented. If these mice are killed at different intervals after inoculation and a histological study is made of the implants and the surrounding tissue, it is found that the tumor grows for a

period of six or seven days as readily as in the Japanese waltzing mouse, the variety in which it originated and in which implants invariably continue to grow. During this time the common mouse provides stroma and blood-vessels for the implant. At the end of this period, an inflammatory reaction appears evidently as the result either of a slight injury to the surrounding tissue or of some chemotactic substance, and the cellular exudate accumulates in amount sufficient to interfere with the blood supply and to isolate the tumor from all healthy supporting tissue so that its destruction is accomplished. If common mice in which this reaction has taken place are subsequently reinoculated with the same tumor, the inflammatory reaction appears several days earlier and the implant is thus disposed of much more promptly than in similar untreated common mice. Such non-susceptible common mice are not, therefore, strictly speaking, naturally immune, but develop an immunity which first manifests itself six or seven days after inoculation. They thus develop a quality not present in the untreated mice, in other words an *active immunity*.

*Non-susceptibility versus immunity.* To ascribe non-susceptibility to "natural immunity" is confusing if not inaccurate, for there is ample evidence to show that this quality is based on an ability to acquire, under artificial conditions, an active immunity. Russell states that the natural resistance of animals may be considered as nothing more than an ability to develop readily an active immunity. It appears preferable to use the terms *non-susceptibility* and *susceptibility*, the former to indicate ability to develop effective immunity to a given tumor so as to make its continued development impossible, the latter to indicate failure to develop effective immunity in response to an implant of tumor. The two terms find application with respect to both individuals and races. While Japanese waltzing mice furnish an example of an uniformly susceptible, and common mice of an uniformly non-susceptible race, with respect to a single tumor, with other races and other tumors varying proportions of susceptible and non-susceptible individuals are found. There are various degrees of susceptibility, and this character is also subject to variation

in individuals, since certain animals in which tumor implants would ordinarily grow may be rendered immune and thereby non-susceptible by the injection of normal tissue or poorly growing tumor. Other animals which are non-susceptible may be made non-resistant through appropriate treatment.

*Hypotheses in explanation of tumor immunity.* Since active immunity to implanted tumor is recognized as an established fact, the question next arises as to the nature of this immunity. A number of hypotheses have been offered in explanation.

The body fluids of immune mice have not been observed to have any direct cytolytic action on tumor cells either in the test tube or in the body of the immune animals. Immunity to homoioplastic implants thus appears to differ from that produced by the injection of the tissue of a foreign species in which cytolysins, precipitins, opsonins, etc., are readily demonstrated.

The possible importance of inter-current bacterial infection in tumor immunity has been emphasized by Pitzman, who claims that infected tumors confer protection whereas non-infected tumors do not. This is not in accordance with many well established facts, and it is especially difficult on this basis to account for the constant appearance of an inflammatory reaction around implants in one variety and its absence around implants in parallel series of another variety of mice. It is likewise difficult on the hypothesis of immunity through concomitant infection to explain the occurrence of an inflammatory reaction only around living tumor, its prompt subsidence as soon as the latter becomes wholly necrotic, and also the failure of necrotic tumor to produce immunity. In order to put this hypothesis to further test a tumor infected with a non-pathogenic bacillus was ground in salt solution and filtered through filter paper. The filtrate was inoculated subcutaneously into a series of mice and later these, together with an equal number of controls, were inoculated with tumor contaminated with the same bacillus. Implants taken twenty and twenty-six hours after inoculation showed in the treated mice more polymorphonuclear leucocytes in foci around the tumor than was the case in the controls. After forty-eight hours, however, the implants in the treated animals showed prac-

tically no infiltration and were developing under more favorable conditions than the controls. These findings are not, therefore, in accord with the view that tumor immunity is brought about through bacterial infection.

Athrepsia, or deficiency on the part of the body fluids with respect to certain substances essential for the nutrition of the tumor, fails to account for both the vigorous temporary growth in non-susceptible animals, and also the inflammatory reaction which precedes the destruction of the tumor.

The hypothesis that something is produced in the immune animal which renders inert the products of the tumor and so inhibits the proliferation of stroma and blood-vessels has been advanced by Russell and others. While this would not account for the retrogression of large established tumors, we have no evidence to disprove that this mechanism applies to certain cases in which there is no active inflammatory process about the implant. Theoretically, it would appear quite probable that, in instances where the tumor cells were less foreign in nature, the immune body produced may call forth no excessive reaction on the part of the host tissue and nevertheless be sufficient to inhibit the stimulating effect of the tumor on the growth of blood-vessels and connective tissue.

From the observations already alluded to, it appears evident that a large proportion of tumor implants in non-susceptible mice are at first provided with both stroma and blood-vessels, but later on become isolated from healthy supporting tissue by an inflammatory reaction manifested by abundant cellular exudation and degenerative changes in the tissue around the tumor. This delayed reaction of the host tissue is difficult to explain except on the hypothesis that an immune body has been produced. Since there is no available evidence indicating the plurality of immune substances in tumor-immune animals, and in view of the failure to demonstrate lysins, precipitins, or any of the recognized immune bodies, it appears justifiable for purposes of discussion to speak of the protective material present as a single substance or immune body. With an immune body present, the tumor products are rendered strongly chemotactic so that the

surrounding tissue, as well as the implant, becomes infiltrated with leucocytes. The reaction is not merely *exudative* in character but is *proliferative* as well, for there is a great increase in the number of fibroblasts in the surrounding tissue, and these increase in size as in granulation tissue. That others have attached so great importance to lymphocyte infiltration may be due in part to difference of material, to the period at which the process was studied, or to a failure to recognize all the constituents of the reaction. From both the material on which the present study is based, and from the illustrations of other authors, it is evident that we have in general to deal, not with an infiltration of a single type of cell but with an inflammation which may vary somewhat with the material though more especially with its duration. Thus in immunized mice, the reaction is more prompt and polynuclear leucocytes are more numerous, while in untreated nonsusceptible mice the reaction appears later—not earlier than the seventh day—and here the lymphocytes occur in greater numbers. In both instances the reaction manifests itself in the formation of granulation tissue in which proliferative as well as exudative activity is evident.

The pale staining and vacuolation of the cytoplasm, the swelling of the nuclei, and the absence of cell division are taken as indices of degeneration in the tumor epithelium. Such degenerative changes are of frequent occurrence in implanted tumor epithelium, even in susceptible animals, and they usually occur in those portions of the epithelium farthest from the host tissue, i.e., from the source of nutrition. From the fact that the features of tumor cell degeneration are the same in both susceptible and immune animals, from the gradual progress of this degeneration, and from the visible evidence of a reaction which tends to isolate the tumor from healthy tissue, it is evident that the destruction of the tumor is accomplished by the formation of an immune body which modifies the response of the host tissue.

The number of lymphoid cells in the inflammation around the tumor implant was regarded as significant by Da Fano who has concluded that it is through the agency of the lymphocytes that immunity is produced. Murphy has arrived at similar

conclusions after having found that a rat tumor may be grown in chick embryos in the absence of lymphoid tissue, and that the introduction of certain adult tissues, such as spleen or lymph-node, is sufficient to prevent this. Baeslack has shown a relative increase in the number of lymphocytes in the blood in the course of the retrogression, and a relative decrease during the active growth of tumors. Murphy and Morton, who have made a study of the blood counts in artificially immunized, naturally non-susceptible, and susceptible mice, have also demonstrated a very marked absolute increase in the number of lymphocytes during the retrogression of implanted tumors. In neither of these articles has the possibility of differences in the leucocyte content of the blood of the tail vessels and that of the heart in rats and mice been discussed. That the white count from the blood of the tail vein may vary within wide limits has been pointed out by Klieneberger and Karl and this has been the experience of the author. Since the white count of the blood of the tail vein may vary more than 100 per cent and is usually much higher than that of the heart's blood, and since differential counts show that the lymphocytes are approximately 20 per cent more numerous in blood from the tail, the indicated increase in the number of lymphocytes in the blood of immune mice is probably greatly exaggerated. The charts presented by Murphy and Morton show no increase in the lymphocyte count of the immunized mice except on the introduction of tumor tissue. Thus the lymphocyte count in the immunized mouse is shown at precisely the same level before and after the immunizing injection, and yet it would not be denied that an animal so treated now possesses qualities which were previously absent, or in other words had become immune—a point of considerable significance. This, together with the fact that these authors were able to lower the resistance of immunized animals by exposure to the X-rays, which are known to have a destructive action on lymphoid cells and other leucocytes, lends additional support to the hypothesis already outlined, i.e., that resistance to transplanted tumor is dependent upon (1) the presence of an immune body which, in the presence of the tumor, either produces injury or otherwise

renders the implant chemotactic, and (2) an ability of the tissues to respond to this with an inflammatory reaction which isolates and destroys the implant.

Although it may be possible by repeated X-ray radiation in appropriate amounts to render both naturally non-susceptible and artificially immunized animals favorable for the growth of implanted tumor, there is in the physiological mechanism thus destroyed nothing specific to tumor immunity. It is inconceivable that there should be sufficient differences in the lymphocyte content of various classes of mice to account for their differences in susceptibility to a given tumor. In fact, other publications by one of these authors tend to show that X-ray radiation tends to lower resistance to a variety of disease-producing agents. The X-ray may evidently destroy the ability of the organism to respond to a certain type of injury with a corresponding type of inflammatory reaction and, applied to tumor immune animals, it in this way decreases their resistance.

It thus appears most probable that at least three factors are concerned in the process of the elimination of implanted tumor. First, antigen which diffuses from living tumor cells; second, antibody which in the presence of antigen renders the implant positively chemotactic; and third, in response to this an inflammatory reaction. With the formation of this immune body tumor products which had previously produced a mild proliferation of the surrounding host tissue are now made strongly chemotactic to leucocytes, and probably slightly injurious to the surrounding tissue. Whether another substance such as complement is necessary in this reaction has not been demonstrated. With the material at hand there is no evidence of a specific chemotactic influence on the lymphoid cells, for different varieties of wandering cells are attracted and other cells stimulated to proliferate. The character of the inflammatory reaction varies here as elsewhere both with the degree of excitation and with the duration of the process. The lymphoid cells preponderate in the milder and more prolonged reaction, polymorphonuclear leucocytes and endothelial phagocytes in the more prompt and pronounced reactions, and the reaction of the connective tissue is also an important factor.

*Passive immunity.* Following the recognition of the immunity of mice in which large implanted tumors had retrogressed, attempts were made to produce passive immunity and through the use of the serum of such immune mice to cure other mice of their tumors. Although success was at first reported, a further trial of such supposedly immune serum was attended with no more frequent retrogression than occurred naturally. In the light of the difficulty in curing established tumors, it is remarkable that no greater effort has been made to demonstrate passive immunity with respect to subsequently implanted tumor. While Gaylord, Clowes and Baeslack found that the treatment of mice with immune serum appeared to have the effect of lowering the number of takes from subsequent implants, Weil found that the similar treatment of rats with immune serum failed not only to influence the growth of established tumors, but also to prevent the growth of subsequent implants of sarcoma. He was also unable to demonstrate the presence of an immune body by passive sensitization of guinea-pigs with the serum of tumor immune rats. In this instance both the immune plasma and the tumor were derived from a single species, the rat, so that less pronounced results might be expected than in immunity to a foreign proteid. In fact, these results are not inconsistent with the presence of an immune body which influences the reaction of the host but does not directly destroy the tumor cells. It has appeared probable that by histological study reactions may be detected which are not demonstrable in the gross phenomena attending the experiment. The object of the following experiment was to ascertain whether the injection of immune serum at the time of the implantation of tumor in susceptible mice would prevent or in any way influence growth and also whether this in any way modified the reaction of the host tissue to the implant.

*Experiment*

With a view of conferring a passive immunity to carcinoma J. w. A on Japanese waltzing mice, an immune serum was obtained from non-susceptible mice which had been previously immunized by implants of Japanese waltzing mouse tumors. The group of non-susceptible mice employed consisted of eight $F_2$, two $F_3$, and seven $F_4$ hybrids which had

been inoculated on July 27, 1915 with sarcoma J. w. B with negative result. These were inoculated again on Oct. 11, 1915, with carcinoma J. w. A. On bacteriological examination of this tumor no organism was found either in smear or culture. Fifteen days later—Oct. 26—the blood of these mice was collected and defibrinated. With the exception of one mouse which showed a consolidation of a portion of the lung, all appeared in a healthy condition. The blood was kept at a low temperature for about one hour, then centrifugated and the serum used immediately. That the serum was somewhat tinged with red may possibly have been due to pooling the bloods of so many animals.

October 26, 1915. Thirty-six Japanese waltzing mice were employed for the test and these were grouped in three series and treated as follows.

Series I. Twelve controls each received an implant of tumor J. w. A. beneath the skin just posterior to the fore leg.

Series II. Twelve mice received each a subcutaneous injection of 0.3 cc. of the immune serum and immediately following this an implant of tumor J. w. A. The serum was injected into the back just anterior to the tail, while the tumor was implanted behind the fore leg as in the controls.

Series III. The bits of tumor used to inoculate the remaining twelve mice were first placed for about one hour in the small amount of serum left over from the preceding series.

At fixed intervals an animal from each of the three series was killed in order to obtain the tissues for histological study. Six from each series were killed and the others left, to observe any differences in the growth of the implants. (A non-pathogenic bacillus was found in cultures from the tumor here employed.)

Although the implants were smaller at the end of the first week in certain mice of the two series in which immune serum was used, the subsequent rate of growth of the tumor was more rapid in these than in the controls, so that at the end of four weeks all were larger than the largest tumor of the control series. Since the numbers were small, these results are only significant in showing that the immune serum administered at the time of implantation did not prevent the development of the tumor in susceptible mice, and that after a short interval it may have had a stimulating rather than a retarding effect on its growth.

No marked histological differences in the reaction of the tissues of the animals of the control series and of those which had received serum-soaked tumor were observed. Readily distinguishable differences with respect to reaction to the tumor implant were noted, however, in the animals treated with immune serum. The tumor epithelium of the 24 and 26 hour implants in the serum-treated series showed few mitotic figures, although these were numerous in the control implants at this time. There was in all implants of the treated animals an extensive degeneration of the tumor epithelium and the living remnants were scattered in isolated islands, whereas in the controls the living tumor formed a more or less continuous peripheral layer. The swelling, pale staining, and vacuolation of cells may be taken as evidence of degeneration, since such changes are ordinarily found in the living portions of implants bordering on the necrotic interior and farthest from the source of nutrition. Although the degeneration of the implanted tumor in the treated mice appeared to be of the same general character as in the untreated, it was more pronounced in the former, and this difference with respect to the extent of the degeneration increased for at least five days. Infiltration of the interior of the implant with polymorphonuclear leucocytes appeared early—twenty-six hours—and persisted for at least four days. The interior of the control implants, although necrotic, was not in any case markedly infiltrated. For three days the amount of collagen in and around the implants was the same in all three series. There was, however, a marked excess of this material within the four and five day implants of the serum-treated mice. The tumor appears to require a somewhat cellular connective tissue for its growth, and the abnormal intercellular substance which appeared in the serum-treated mice was evidently unsuitable in this respect.

From these observations it is evident that the injection of immune serum modified the reaction of the tissues of the implant and host, although not sufficiently to prevent the later development of the tumor. It is possible that the immune serum injected contained a constituent which was slightly toxic for the tumor epithelium, as was indicated by the absence of mitotic

figures for a time, and by a degeneration which continued for several days, although it is to be noted that similar degenerative changes were commonly found in portions of implants at a distance from the source of nutrition. It is clear, however, that the immune serum has rendered the implant, especially its necrotic and degenerating portions, positively chemotactic to polynuclear leucocytes. Appearing somewhat later—four days after inoculation—there was an increase of collagen within the implant, and phagocytic cells also appeared. The implanted tumor thus encountered in the serum-treated animals conditions which, although transitory and not sufficient to accomplish passive immunity, were nevertheless definitely unfavorable to its development. It should be noted that the transplantable tumor for which the immune serum was prepared encounters conditions in the Japanese waltzing mouse unusually favorable for its growth so that it is not improbable that effective passive immunity with respect to subsequently implanted tumor may be produced by employing other material.

*Specificity.* The fact that blood and other normal tissue may be employed as well as tumor tissue in the production of immunity to the latter, indicates that there is no marked degree of tissue specificity in the immunity which develops, but it is probable that there is a considerable degree of racial specificity with respect to the genetic origin or foreignness of the antigens. That certain tumors, however, show greater differences in their immunizing qualities than the embryonic tissues from which they have arisen will be shown later on by the results obtained in the transplantation of hybrid tumors to the parental stocks.

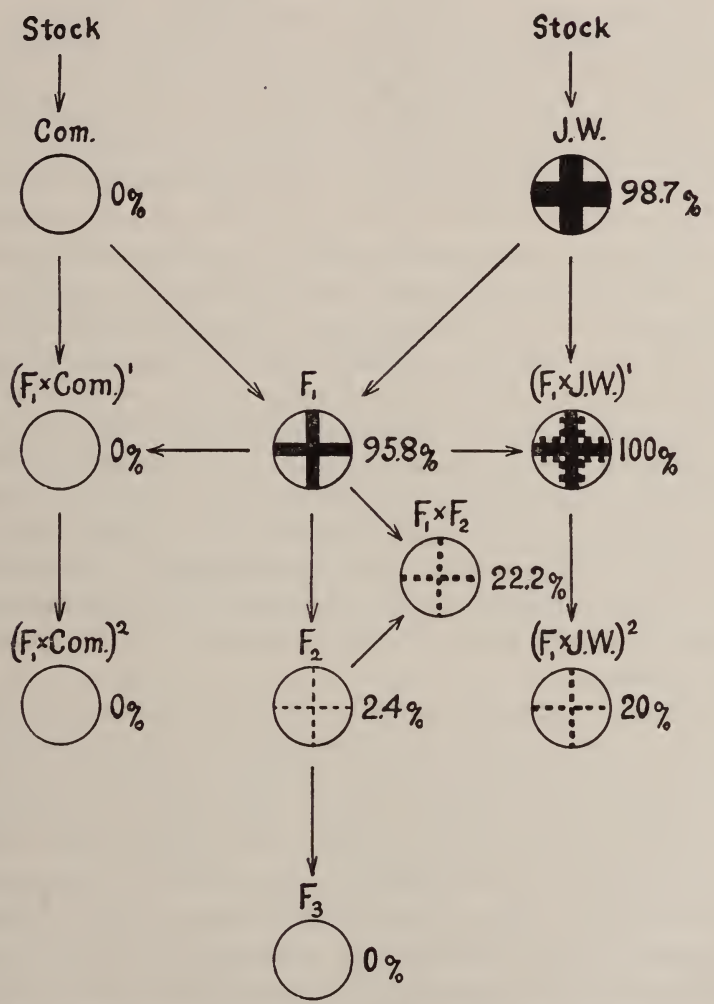
*Foreignness of tumor with respect to host a requisite of tumor immunity and an explanation of the inheritance of susceptibility to implanted tumor.* A carcinoma which originated in the Japanese waltzing mouse is found to grow in practically every individual of this variety in which it is implanted. On the contrary, it fails to grow in every instance on implantation in the common mouse. By cross-breeding these two varieties successive generations of hybrids have been obtained, the first of which has been back-crossed with both parent stocks. The results obtained from

the implantation of this tumor into mice of the various filial generations, and into mice derived from all other matings, are shown in the accompanying chart. The susceptibility of each class has been tested by the simultaneous inoculation of comparative series of mice with a single tumor. The material here considered collectively includes that which has been previously presented by the author, as well as that collected recently in collaboration with Little.

It is quite apparent from these data that susceptibility is not inherited as a single mendelizing factor, for both in the successive filial generations and in the back-crosses between the first filial generation and the parent stocks, the results do not furnish a ratio characteristic of single factor inheritance. There is no blending inheritance, for the results are not intermediate but correspond closely with those obtained in one or the other of the parent stocks. The only hypothesis upon which we can explain these results is, that susceptibility, or non-susceptibility is dependent upon the presence of a complex of independently inherited unit factors. Upon the number of factors necessary for susceptibility will depend the results obtained in the second filial generation. If a few factors are necessary, a considerable proportion of the individuals of this generation should prove susceptible; if a larger number of factors are necessary, then a smaller proportion of the individuals of this generation should be susceptible. In the $F_1$ hybrids, all the inheritable factors of each parent will presumably be present in a single representation. On the principle that these factors will be segregated in the germ cells of this generation, it is possible to compute the results of the random combination of any number of factors which will occur in the mating of the $F_1$ animals. Although the nature of the material employed makes large numbers requisite for an accurate estimation of the number of factors involved, the results obtained indicate that susceptibility to this tumor is dependent on the presence of a large number of independently inherited factors. Our results with the two stocks of common mice recently employed are such as would be obtained if the presence of from twelve to fourteen such factors were necessary for susceptibility.

CHART 1

## SUSCEPTIBILITY TO CARCINOMA J.W.A.




| | | | |
|---|---|---|---|
| $F_1$ from Lab. Stock | — 98.5% | $F_2$ from Lab. Stock | — 5.0% |
| " " Br.Ag. " | — 100.0" | " " Br.Ag. " | — 1.7" |
| " " d.Br. " | — 95.3" | " " d.Br. " | — 1.4" |
| " " Alien " | — 69.2" | | |

*The effect of a single representation of factors on susceptibility.* Although the factors from both susceptible and non-susceptible parent stocks must necessarily be present in the $F_1$ hybrid, it is apparent that those necessary for susceptibility, although here only singly represented, are in the greater part of our material as effective as when doubly represented in the Japanese waltzing mouse. Conversely, the factors necessary for non-susceptibility, although all present in the same $F_1$ hybrids, are ineffective when singly represented or in half dose. That this will not hold true in the cross-breeding of every stock of the common with the Japanese waltzing mouse is clearly indicated in certain earlier experiments. The difference between the percentage of positive results obtained in the $F_1$ hybrids and in the Japanese waltzing mice in recent experiments might possibly be attributable to faulty technic, since only one $F_1$ animal (1.6 per cent) failed to grow the tumor. In a previous investigation a slightly higher proportion of failures occurred, and the fact that most of these were in $F_1$ hybrids ("Alien" $F_1$ hybrids) which were derived from another stock of common mice indicates that a single representation of the factor complex of non-susceptibility may in some instances prove effective. The non-susceptibility of certain of these negative $F_1$ hybrids was established by the negative results of reinoculation.

*The effect of single representation of factors on the rate of growth.* Notwithstanding the presence of the factors of non-susceptibility in the $F_1$ hybrid, the rate of tumor growth is usually more rapid than in the Japanese waltzing mouse. This fact was established in the earlier experiments by weighing the tumors after equal periods of growth in a comparative series of mice. Subsequent experience has also shown that the tumor grows, on the average, more rapidly in the $F_1$ hybrids. It is probable that this increase in the rate of tumor growth is due to certain factors furnished by the non-susceptible parent stock. While we are unable to recognize the individual factors concerned, it might be expected that the more rapidly growing and larger hybrids supply the tumor with more abundant nutrition than is the case with the more slowly growing and smaller Japanese waltzing mice. It is

found, however, that the tumor grows more rapidly than in Japanese waltzing mice, not only in young growing but also in full-grown $F_1$ hybrids. The presence of certain singly represented factors of non-susceptibility evidently calls forth a greater growth of stroma and blood-vessels, so that they in this way appear to have a stimulating effect on the growth of the tumor. The injection of the serum of immunized mice also has been shown to have a transitory retarding but a later stimulating effect on the growth of tumor J. w. A. in the susceptible Japanese waltzing mouse.

*Factor representation in retarded growth.* Although in recent experiments, 3 of 183 hybrids of the second filial generation have shown progressively growing tumors, the rate of growth has been much slower than in the Japanese waltzing or $F_1$ hybrid mice. From this it appears probable either that not all of the factors found in the Japanese waltzing mice are present even in a single representation, or that certain of the factors of non-susceptibility become effective through double representation in these positive $F_2$ mice, for otherwise there is nothing to account for a rate of growth so comparatively slow. In addition to these individuals in the $F_2$ generation in which the tumor grew progressively, there were certain others in which the tumor grew for a time but eventually disappeared. For these it is necessary to assume a still more limited representation of certain factors with a correspondingly greater representation of others.

These results having been interpreted from the viewpoint of genetics, it is now important to consider them in the light of what is established for tumor immunity. It has already been pointed out that non-susceptibility of a class of animals to implanted tumor is to be regarded as an ability to acquire an active immunity, and conversely, susceptibility as an inability to develop active immunity to a given tumor. If a single tumor be employed as a constant with which to test various groups of mice, it may be found that the race in which it originated is susceptible whereas another race is non-susceptible. Non-susceptibility is thus based on *foreignness* or *unlikeness* with respect to races, so that when the tissue of one is introduced into the other

active immunity is developed. The science of genetics has already established a series of independently inherited unit factors for a considerable number of species, including the mouse. In the consideration of foreignness or unlikeness, what else could be possible than that, in the comparison of individuals or races, some should differ with respect to few factors and others with respect to many factors? Thus the conception of foreignness or unlikeness not only furnishes a basis for tumor immunity, but also makes more comprehensible the inheritance of susceptibility to implanted tumor.

*Foreignness as a basis of certain biological differences in tumor.* If groups of mice of known character are used as constants with which to test various tumors, differences in the behavior of the latter are detected, even though they may have arisen in a single inbred race. It assists materially in the explanation of results if these biological differences are recognized. Certain tumors occur which are transplantable in only a small proportion of cases, even in closely related animals. The conditions requisite for their growth are found rarely apart from the individual in which they arise, i.e., a slight degree of foreignness on the part of the host tissue is fatal to them. For other tumors fewer conditions or factors are necessary, and such, since they are able to withstand a higher degree of foreignness on the part of the host, develop on transplantation in a greater proportion of cases. Such differences in the capacity of a number of tumors for growth in various classes of mice are shown in the following table.

The biological difference or foreignness of tumors with respect to one another and to the normal somatic tissue from which they arise, is a point of considerable significance. To illustrate: a tumor (H. F.) which arose spontaneously in an $F_1$ hybrid has been implanted into other individuals of the same generation, and into the parent stocks of common and Japanese waltzing mice. Now the animal in which this tumor appeared grew as an embryo upon the uterine mucosa of its common mother, without the appearance of any incompatibility between the embryonic and maternal tissue. Since the tumor which has arisen from the somatic tissue of this mouse fails to grow in either of

the parent stocks, it would appear probable on this basis that it differs from the soma with respect to its ability to grow on a given soil. Loeb, however, has already called attention to the absence of antagonistic reaction in utero between the maternal and the somewhat foreign embryonic tissue. This $F_1$ tumor appears to have required factors for its growth which were not all furnished by either the common or the Japanese waltzing mouse nor by all $F_1$ hybrids. It might be expected that in the cells of this tumor, as in other somatic cells of the $F_1$ hybrid, there would be an equal representation of factors of both parents. The only alternative would be to consider the hybrid tumor as a manifestation of mosaic inheritance and thus corresponding more closely in its character to the soma of one or the other parent stock. If such were the case, however, it would be expected that this tumor would grow in one of the parent stocks, at least as well as in the $F_1$ hybrids; but the results are otherwise. The Japanese waltzing mice in which it failed to grow were all found to be susceptible to tumor J. w A., J. w. B. or J. w G., so that its failure is to be attributed to feeble growth power, i.e., inability to grow in the presence of even a few foreign factors, rather than to any unusual immunizing property.

Another tumor (H. G.) which arose in a back-cross hybrid from the mating of an $F_1$ hybrid with a common mouse, failed to grow in common as well as Japanese waltzing mice, although closely related to the former.

Differences in behavior with respect to transplantability are found not only in tumors arising from heterogeneous stocks, such as the Jensen and Ehrlich *Stamm* 11 carcinomata, but also in tumors arising in inbred stocks such as the Japanese waltzing mouse. The behavior of the tumors arising in the $F_1$ hybrids can not be accounted for on the basis of characters derived either from one of the parents or from both, but only on the basis of the appearance of modifications or new characteristics.

From the evidence in the biological character of tumors of a permanent modification of somatic tissue, it appears logical to regard a tumor as a manifestation of *somatic mutation*. As a basis for this, there may be modification in the relative value

CHART 2

| TUMOR | COMMON TAME MOUSE | JAPANESE WALTZING MOUSE | $F_1$ HYBRID | $F_2$ HYBRID | $F_3$ HYBRID | $(F_1 \times Com.)^1$ HYBRID | $(F_1 \times Com.)^2$ HYBRID | $(F_1 \times J.w.)^1$ HYBRID | $(F_1 \times J.w.)^2$ HYBRID | $(F_1 \times F_2)^1$ HYBRID | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Common mouse Carcinoma (Jensen) | ± 9+ 1− | — 9− | ∓ 9− 1+ | | | | | | | | Differences in susceptibility of various races of common mice noted but data not included. |
| Common mouse Carcinoma (Ehrlich) | ± 15+ | ∓ 5− 3+ | ± 5+ 5− | | | | | | | | Differences in susceptibility of various races of common mice to Ehrlich tumor also noted. |
| Japanese waltzing* mouse, Carcinoma (J. w. A.) | — 147− | ∓ 893+ 12− | ± 126+ 2− (Alien) 9+ 4− | ∓ 237− 6+ | — 71− | — 78− | — 34− | + 63+ | ∓ 4− 1+ | ∓ 7− 2+ | Eleven of twelve negative J. w.'s not reinoculated. Three of alien $F_1$ hybrids not reinoculated. |
| Japanese waltzing† mouse, Sarcoma (J. w. B.) | — | + 185+ | + | ∓ | | | | | | | Susceptibility of other classes to this tumor now under investigation so that data are not included. |
| Japanese waltzing mouse, Sarcoma (J. w. G.) | | + 28+ | | | | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $F_1$ Hybrid Carcinoma (H. F.)† | — 8— | — 13— | ∓ 12— 3+ (Alien) 4— | — 11— | | | | | | | Subsequent implantation of carcinoma J. w. A. (9), Sarcomata J. w. B. (7) and J. w. G. (5) in the same J. w. mice was successful in every case. Four negative $F_1$ hybrids were found susceptible to Sarcoma J. w. B. |
| $F_1$ Hybrid Carcinoma (H. H.) | | — 5— | | | | | | | | | |
| $F_2$ Hybrid Carcinoma (H. D.) | — 5— | | | — 14— | | | | | | | |
| ($F_1$ × Com.)[1] Hybrid Carcinoma (H. G.)† | — 11— | — 11— | | | | | | | | | This tumor evidently produces no immunity to Sarcoma J. w. B. which grew in 4 of these J. w. mice subsequently inoculated. |
| Hybrid? (presented by Dr. Gaylord) Carcinoma (H. C.) | | — 9— | | — 6— | | | | | | | |

* See references Tyzzer, and Little and Tyzzer.

† Now under investigation by the same authors.

either by loss or addition, or in the nature of factors, any of which, if continuously transmitted thereafter in successive cell generations will constitute a type of mutation. This, unlike the mutations which may affect the germ plasm, is maintained only through artificial transplantation from one individual to another. The tissue of a new growth has thus in certain respects become foreign to the other tissues. Its growth is no longer controlled by the normal inhibiting influences which constitute a regulating mechanism, but it behaves more or less as a parasite living at the expense of its host; and it may excite a reaction of the surrounding tissue which is in some cases more favorable, in other cases less favorable, to its continued growth. Malignant tumors must have feeble antigenic power as well as sufficient resistance to the normal inhibiting influences to provide for continued growth in the animal in which they originate, otherwise reactions sufficient to destroy them would occur more frequently.

*Conclusions.* The results of the experimental investigation of tumors, as well as of clinical and pathological observation, appear to favor the following conception of the nature of tumors and their relationship to the other tissues.

The inter-reactions of the normal tissues are mutually beneficial so that their relationship is one of symbiosis.

The anomalies and benign growths, while not distinctly harmful, are usually of no benefit to the individual; the relationship is one of commensalism.

The malignant tumors are in many respects parasitic in nature, especially since they develop at the expense of the other tissues of the body. They are so adapted for growth, once they have become established, that they seldom arouse any effective resistance on the part of the body. There is some evidence, however, of a local reaction of tissues unfavorable to the growth of many different types of tumors.

Immunity to transplanted tumor is based on foreignness or incompatibility of tumor and host. This holds true whether the tumor or the animal is taken as the constant factor with which to test the other. Although the degree of foreignness is not sufficient for the production of markedly cytotoxic or cytolytic

sera, as when different species are employed, it appears probable that an immune body is formed which, in the presence of the antigen—or living tumor—excites an inflammatory reaction in the tissue around the tumor so that the latter is isolated and eventually destroyed.

Both susceptibility and non-susceptibility, or the ability to acquire immunity, are inherited, not as a single unit factor but apparently as a complex of mendelizing factors. Non-susceptibility and susceptibility are apparently based on factor differences, or, in other words, on unlikeness or foreignness. Non-susceptibility may thus depend with one tumor on a difference with respect to few factors, and with another tumor on a difference with respect to many factors. In the comparison of a stock of Japanese waltzing and several stocks of common mice, the non-susceptibility of the latter to a carcinoma, J. w. A., is based on a difference with respect to a large number—probably twelve to fourteen—of independently inherited factors.

Susceptibility is in this material a dominant character, since it is manifested when its factors are present in a single representation, as in the $F_1$ hybrid. The presence of a single representation of the factors of non-susceptibility in the $F_1$ hybrid apparently stimulates the growth of the tumor, for its rate of growth is more rapid than in the Japanese waltzing mouse in which the factors of susceptibility are doubly represented.

There are marked differences in the behavior of various tumors on transplantation in given classes of mice. Even tumors arising in homogeneous races show such differences, and this may be attributed to the acquisition of new characteristics by the soma which are manifested in the development of the tumor. The tumor, since it breeds true with respect to these characteristics in the course of artificial propagation, may be regarded as a modification of the somatic tissue which may be termed *somatic mutation.*

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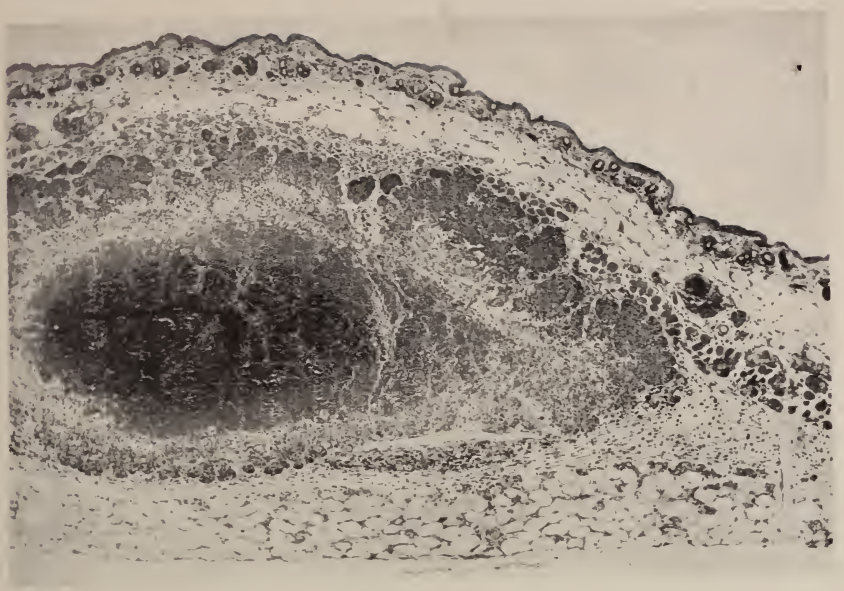
## DESCRIPTION OF PLATE 1

FIG. 1. Carcinoma (J. w. A.) 98 hours after implantation in a normal control Japanese waltzing mouse (no. 5417). Living tumor is distributed at the periphery, forming a layer with only occasional breaks in its continuity. The necrotic central portion of the implant is free from infiltration.

FIG. 2. Carcinoma (J. w. A) 98 hours after implantation in a Japanese waltzing mouse (no. 5423) which was injected with immune serum. Living tumor is found in scattered islands and is absent from large portions of the implant's surface. The necrotic interior shows extensive infiltration with polymorphonuclear leucocytes.



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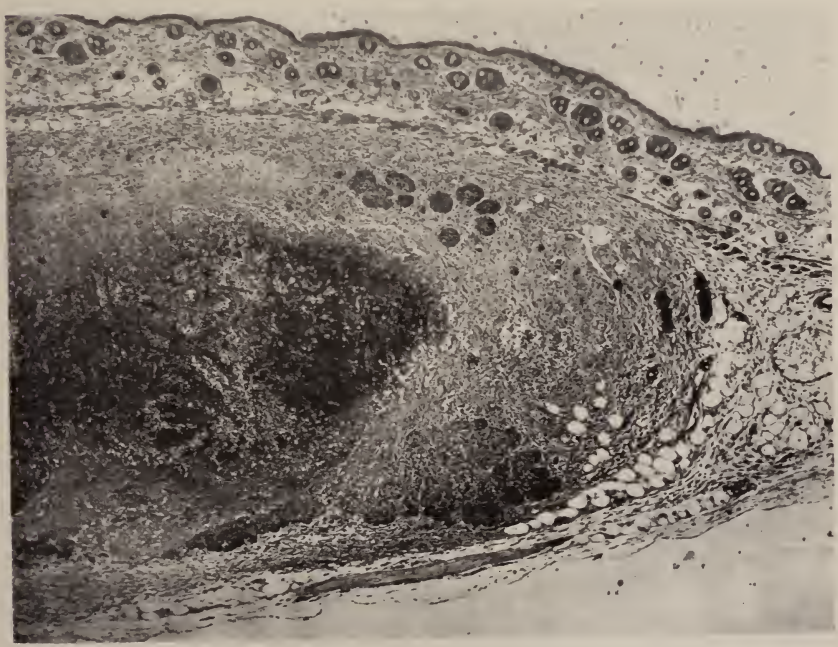

2

# RENAL TUMORS IN THE RABBIT

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New growths of any kind are evidently rare in rabbits. Although the rabbit is very extensively used for laboratory purposes, we have found reports of only thirty-five tumors. Twenty-four of the recorded tumors were uterine. Of these Stilling and Beitzke (1) have reported thirteen cases, noting a distinctly hereditary tendency to tumor formation. The youngest animal in their series was four years old. Boycott (2) has recorded four cases, and Wagner (3), Selinow (4), Katase (5), Marie and Aubertin (6), Leitch (7), and Shattock (8) have found one each. Shattock and Leitch both agreed that the case described by Lack (9) was really a primary uterine tumor with metastases. Schmorl (10) reported one case of carcinoma of the lung and a second case with carcinoma of the stomach. Von Dungern (11) and Baumgarten (12) each described a case of sarcoma, while Schultz (13) has done some very interesting work with a transplantable round cell sarcoma. Petit (14) has recorded a primary carcinoma of the lung and a carcinoma of an accessory pancreas in the omentum, and Bashford (15) has found a carcinoma of the mamma and a sarcoma of the subcutaneous tissue.

Of the thirty-five tumors recorded, only two were tumors of the kidney. The first, described by Lubarsch (16) in 1905, was similar to the two that we are reporting. In Lubarsch's case the growth occupied the upper half of the left kidney, and was sharply marked off from the renal tissue. Upon microscopic examination it was found to be composed mainly of gland-like structures of varying form and width, embedded in a very cellular stroma. Lubarsch noted that the histological structure bore

a close resemblance to the human renal tumor commonly described as an adenosarcoma; no cartilage, squamous epithelium, or striated muscle were found, and no Malpighian corpuscles were mentioned. Pieces of the tumor were inserted into the kidneys of four rabbits, with negative results. The kidney had been previously inoculated with embryonic salivary gland tissue but the author did not believe that the tumor grew from this implant; he thought, however, that the injection might have stimulated tissue rests to growth.

The second renal tumor was reported by Nürnberger (17) in 1912. The growth was situated in the upper half of the right kidney and produced a spherical smooth elevation of the surface about the size of a cherry; a sagittal section through the organ showed a tumor measuring 1.5 cm. by 1.3 cm., and displacing about one-third of the renal tissue. Microscopically, the tumor was composed of numerous cysts of varying size lined with cubical or flattened epithelium, and of large numbers of gland-like tubules lined by high columnar epithelium taking a basic stain and showing many mitotic figures. In some of the tubules the epithelium formed more than one layer; in others there was no lumen, the structure appearing as a solid cord. Between the tubules and cysts there was a cellular connective tissue. Smooth muscle fibers were noted under the capsule of the tumor; no striated muscle, cartilage, bone, or elastic tissue was present. Nürnberger recorded his case as a mixed tumor, and compared it to the embryonal glandular tumors found in man.

Our two neoplasms occurred in adult male rabbits, both having been found on the same afternoon; and although we have autopsied over four hundred rabbits during the last three years, no other tumors have been seen. We were not able to ascertain the ages of the rabbits, neither could we determine whether they were both from the same litter, since animals obtained from different persons had been put into a cage together.

*Case I. Adult male rabbit.* Death was due to surgical shock following an experimental operation. At autopsy, several hours after death, a spherical tumor 1.4 cm. in diameter was found about the center of the outer border of the left kidney (fig. 1).

Apparently it was of cortical origin, since it did not involve the medulla. It was sharply marked off from the renal tissue, fairly firm, and whitish gray in color. A thin prolongation of renal capsule covered the tumor. No metastases were found.

The microscopic structure is shown under moderate magnification in figure 2. The greater part of the tissue consists of cellular masses (*n*) of irregularly rounded shape, in some of which tubules (*t*) are to be seen. They occur in all stages of differentiation from a mere radially arranged cluster of nuclei to a completely formed tubule. The structure of these cellular masses and the fact that they are differentiating into tubules makes it certain that they correspond to the nephrogenous tissue of the embryonic kidney. A similar tissue occurs in a type of human renal tumor which has been described as an adenosarcoma. (Birch-Hirschfeld (18), and Trappe (19)).

In figure 3, an island of nephrogenous tissue is shown in which several tubules have been formed. No connective tissue fibers could be demonstrated in the nephrogenous tissue by Mallory's phosphotungstic acid hematoxylin stain. No mitoses were to be seen in any part of the tumor.

Between the masses of nephrogenous tissue there is usually only coarse connective tissue, but in some areas, as is shown in figure 2, there are a number of irregular tubules lined by flattened epithelium. The nuclei of these cells take a deep hematoxylin stain. These tubules sometimes connect with well developed Malpighian corpuscles. The corpuscle shown in figure 4 has parietal flattened epithelium and a capsular space, and resembles the corresponding adult structure except that there is a complete absence of blood capillaries. A number of well formed corpuscles are to be seen in some sections, and there are a great many incompletely formed. They always lie between the masses of cellular nephrogenous tissue, and none of them contain any blood capillaries. No Malpighian corpuscles are mentioned in the rabbit tumors reported by Lubarsch and Nürnberger, but they have been seen occasionally in tumors of this type occurring in children.

*Case II. Adult male rabbit.* Killed by ether.[1] Autopsy performed immediately afterwards. A large tumor was found at the inner aspect of the caudal pole of the left kidney (fig. 5), not involving the medulla; the inner extremity of the growth was soft and irregular and had ruptured the renal capsule. No metastases were found.

A few small areas of this tumor show islands of cellular nephrogenous tissue such as occurred so abundantly in the first case, but in all other parts the nephrogenous tissue is completely differentiated into masses of solid cords and tubules (fig. 6). Lumina are visible in many of the cords. The greater part of the tissue shows marked retrogressive changes; there is an accumulation of hyaline material in the connective tissue, and the epithelial cords are disintegrating. In some parts, the degeneration of the tissue is so extensive that the epithelial tissues are no longer recognizable as such (fig. 7). No definite Malpighian corpuscles were found in this tumor, and no mitoses.

Rabbits were inoculated with fragments of fresh sterile tumor from Case II, as follows: three subcutaneously, one in the kidney, one in the anterior chamber of the eye, and two intravenously (with emulsified tumor). Negative results were obtained in every case.

The second case may be regarded as similar to the first, though in a much later stage of development. There are a few small areas that closely resemble the structure of the first tumor, but almost everywhere the cellular masses characteristic of the first tumor have differentiated into solid cords and tubules. The growth has ruptured the renal capsule, but it is evidently not very malignant, since there are no mitotic figures and since retrogressive changes have begun in nearly all parts of the growth.

Including our two cases, there are now reports of four tumors of the rabbit kidney. It will be noted that these tumors have

[1] This case affords a strange coincidence. A piece of tumor from Case I had just been transplanted into the cranial pole of the left kidney, when the animal died from the anesthetic. An autopsy performed immediately revealed a large tumor at the caudal pole of the same kidney (this is the tumor described as Case II). No other tumors have been seen by us although the kidneys of over 400 rabbits have been examined during the last three years.

many points of resemblance. Thus, in the gross, they all appeared to be of cortical origin, and were sharply separated from the renal tissue. The only evidence of infiltration was seen in our Case II, in which the renal capsule was penetrated. Microscopically, three of the growths were composed mainly of glandular structures strongly suggesting renal tubules, and the fourth, our Case I, contained a number of these tubular structures. The presence of Malpighian corpuscles is this tumor is convincing evidence that we are dealing with renal tissue.

Our Case I is in some respects the least differentiated of the group, since it contained a large amount of cellular tissue not yet differentiated into tubules; it corresponded closely in structure with the metanephrogenous tissue at the stage when this begins to form tubules. There was a small amount of this metanephrogenous tissue in Case II, but the greater part of this tumor, as well as the tumor reported by Nürnberger, was of tubular structure.

These rabbit tumors thus correspond closely with those neoplasms of the human kidney commonly described as adeno-sarcomata. The simplest interpretation of their origin is to regard them as having developed from portions of the metanephrogenous tissue which became enclosed in the kidney during its early development but failed to form connections with the collecting tubules. Since no striated muscle is present, they are not comparable to the mixed tumors of the kidney which occur so frequently in children, and which are best explained as derived from portions of the primitive segments.

Since there is a commendable tendency at the present time to limit the word sarcoma to malignant growths in which the type cell is a fibroblast, it seems inadvisable to classify these tumors as adeno-sarcomata. They are not mixed tumors as this term implies; the type cells are nephroblasts which grow at first in masses or diffusely without special arrangement (so-called sarcomatous tissue) and later differentiate into tubules. Hence we suggest the term *nephroblastoma* for those tumors in which the type cells tend to form renal tissue only.

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# PLATES

## EXPLANATION OF PLATES

Fig. 1. Case I. Mid-sagittal section of kidney showing tumor. Photomicrograph, × ¾.

Fig. 2. Case I. The rounded cellular masses are islands of nephrogenous tissue, in which tubules may be seen in different stages of formation. Between the rounded masses are numerous tubular structures lined by a low flattened epithelium. Many Malpighian corpuscles are present. (In some areas only the islands of nephrogenous tissue are to be seen.) × 100.

Fig. 3. Case I. Island of nephrogenous tissue under higher magnification. Several tubules are shown. (The nuclei of the tubules are represented much darker than they appeared in the section.) × 300.

Fig. 4. Case I. A Malpighian corpuscle, complete except for the entire absence of blood capillaries. The parietal flattened layer and the capsular space are well shown. Photomicrograph, × 325.

Fig. 5. Case II. Mid-sagittal section of kidney showing tumor. Photomicrograph, × ¾.

Fig. 6. Case II. Island of tubules corresponding to the cellular masses shown in Fig. 2. The connective tissue contains some homogeneous material. × 150.

Fig. 7. Case II. A part of the tumor showing advanced retrogressive changes. The tubules have disintegrated and the connective tissue shows a lar nd. of hyaline material. Photomicrograph, × 300.

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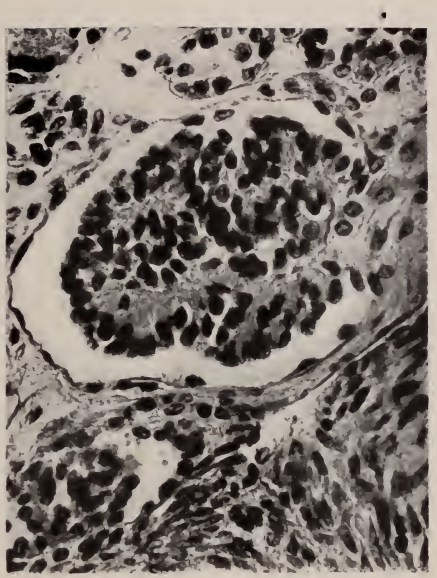

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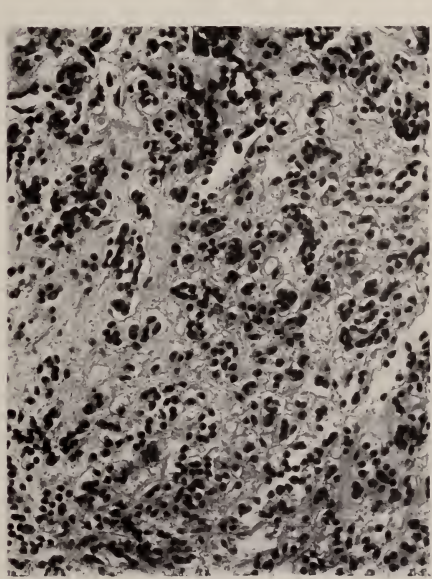

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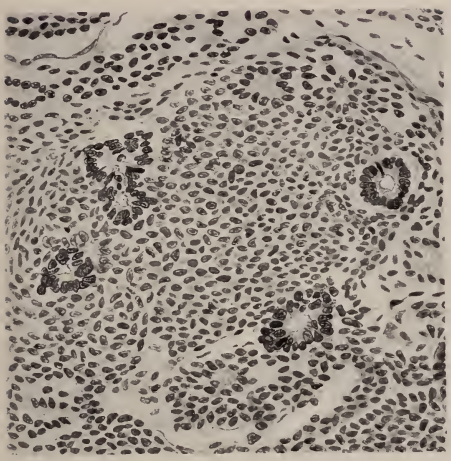

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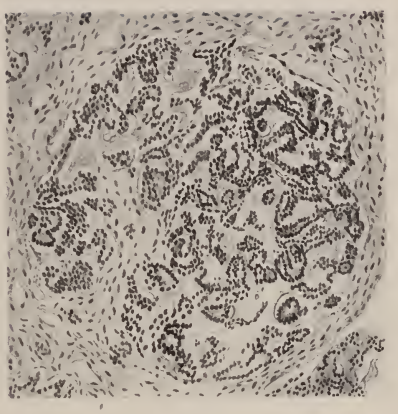

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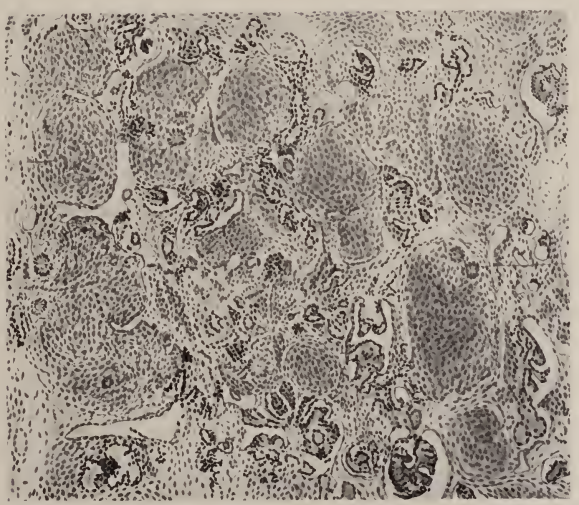

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# TISSUE CULTURES IN THE INVESTIGATION OF CANCER

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It is recognized that progress in many departments of science is dependent in large measure, if not entirely, on the development of improved methods of investigation. In the field of cancer research it has become increasingly evident in recent years that new methods of attack must be worked out before further important advances can be expected.

The discovery of the factors concerned in the unlimited lawless growth of cells constitutes, obviously, the main cancer problem. It would appear therefore that the recently devised method of cultivating tissues *in vitro* (Harrison, Burrows) by which we are enabled to study isolated somatic cells under conditions which allow the introduction of known and measurable factors into experiments, should mark an important advance in the attack upon this fundamental question. Furthermore, since it is possible to subject cancer cells, thus isolated, to various chemical and physical agencies, it may be suggested that we have also in the new method a possible means for solving another great cancer problem, namely, the treatment of malignant growths.

In the study of human tumors, the method will probably fill a great need, since it is not possible to secure the continued propagation of such tumors by transplantation, by the methods pursued in the case of tumors of certain of the lower animals.

The purpose of this paper is simply to review briefly a few of the results which have been obtained with tissue cultures in studies upon cancer and related subjects, and to suggest some problems in which it seems probable that the method may be

advantageously applied. It is possible that a review of the results thus far obtained may prove disappointing. But in palliation it should be said that the method is young and in many respects still imperfect; and that much time and energy have been expended by a number of workers in the past five years toward improving the technique so as to permit the cultivation of a greater variety of tissues over longer periods of time.

Details of the history of the method of tissue cultivation *in vitro* and its development will be found in various papers to which references are given at the end of this article (Loeb (1), Harrison (2, 3), Burrows (4, 5). For the convenience of those interested, a brief review of the technique of the method is given here.

## TECHNIQUE

The simplest and most satisfactory form of culture consists of a small piece of tissue placed in a thin drop of plasma on a cover glass which is inverted and sealed over the cavity of a hollow-ground slide. To obtain the plasma in such a way that it will remain unclotted until the tissue is added, several precautions must be observed. The blood must flow directly into the container (preferably a small test tube) without contamination with tissue juices, and should be kept cold in oiled or paraffined tubes both during and after centrifugation.

Blood is obtained from human beings or large laboratory animals (dogs, goats), by puncturing a superficial vein with a needle of large bore, previously boiled in albolene or olive oil, and allowing the blood to flow directly from the needle into paraffined tubes set in large centrifuge cups which are filled with cracked ice.

Small animals such as mice, rats, and frogs may be bled from the heart in the same way. The writer has devised another method (7), however, for bleeding small animals, which does not entail the sacrifice of the animal as does bleeding from the heart, a distinct advantage in certain experiments where it is important to obtain plasma from the same animal more than once. The carotid, jugular, or some other accessible vessel is exposed and

clamped lightly at two points some distance apart; the vessel wall, after clean dissection, is caught by delicate forceps at a point midway between the clamps in such a way that the lumen is not occluded; the vessel is then severed beyond the forceps, the proximal clamp released and, with the end of the vessel held

FIG. 1. Method of bleeding small animals for obtaining plasma. The artery is held away from the tissues and the blood allowed to spurt into paraffined tubes.

away from the tissues, the blood is allowed to spurt free into cold paraffined tubes as before (fig. 1). Even so small an animal as the mouse may be bled several times in this way.

On account of the dryness of their tissues and the proximity of the wing vessels to the skin, fowls and pigeons may be bled di-

rectly from the wing vein by making a clean incision through the skin and vessel, and allowing the blood to drip from the surface into the tubes.

Blood obtained by any of the methods mentioned is centrifuged at high speed for several minutes, and the supernatant plasma is then put aside in a cold place, or packed in ice.

The length of time that the plasma may be preserved uncoagulated varies greatly with different species. Fowl and pigeon plasma may be kept indefinitely if properly handled. On the other hand, rat and guinea-pig plasma will rarely remain fluid longer than two hours, often not so long. Human, rabbit, dog, and cat plasma may be kept for several hours at least, and sometimes even for days.

The remainder of the technique consists in placing small pieces of tissue, 0.5 to 2.0 mm. in diameter, on a cover glass, and adding immediately a small drop of plasma. The cover glass is inverted without delay over a hollow-ground slide, sealed with vaseline, and incubated at body temperature.

It should be stated in this connection that tissue cells show no such proliferative capacity in cultures as do bacteria. Furthermore, it has not been possible as yet to obtain an active growth of any of the highly differentiated body cells, though certain specialized elements, such as ganglion cells, may survive for a time.

The cultivation of human tissues has offered special difficulties, owing to the fact that the fibrin in the clotted human plasma, which forms the necessary scaffolding for the outgrowth of cells, is regularly digested by the tissue fragments, and the cells, not being able to wander out into the medium, soon die. The writer has overcome this difficulty by using as a culture medium a mixture of human serum (or plasma) and chick plasma, the fibrin of which resists digestion. (It was found that chick plasma alone did not form a satisfactory medium.)

That the method is in many respects surprisingly simple should be emphasized. It has been little used in cancer laboratories, a fact which is probably to be attributed to supposed difficulties in technique (danger of contamination, maintenance of

a uniform temperature during preparation of cultures, etc.), points which have certainly received unmerited emphasis in several papers (6). As a matter of fact, practically no effort at all is required to avoid bacterial contamination; thus, the writer has used tissue from the liver of a human cadaver four hours after death (metastatic tumor nodule lying directly on the colon), and observed not a single colony of bacteria in a large number of preparations. It is possible to use even such a tissue as skin, where a certain number of bacteria are known to be present, if partial sterilization with weak alcohol be first carried out. The plasma itself, especially that of fowls, evidently possesses a definite bactericidal property, by which a certain number of organisms are taken care of. It is not necessary to keep the tissues at or near body temperature while preparing the cultures, and within certain limits the time elapsing between removal of the tissue from the living body and the preparation of the cultures is not important. The tissue of the adult rat may be used after several days' preservation at room temperature or in the ice-box, and the writer has obtained successful growths of human tissue kept for six days in cold storage. Embryonic tissues are capable of longer preservation—five to eighteen days, the length of time depending on the temperature.

The problems in which the method of tissue culture has been employed by the writer may be conveniently discussed under several headings: (1) Comparative study of the behavior of cancer cells and normal cells growing *in vitro;* (2) Cancer immunity; (3) Stimulation of cells growing in cultures.

## COMPARISON OF THE BEHAVIOR IN VITRO OF NORMAL CELLS AND CANCER CELLS

The similarity between the growth *in vitro* of sarcoma and connective tissue on the one hand, and between carcinoma and normal epithelium on the other, has been emphasized in previous papers (7, 15). Sarcoma and connective tissue cells wander out singly or in chains, while epithelial cells, normal or neoplastic, tend to spread out in sheets or groups.

Certain differences which may not be without importance have been observed in the behavior of normal and malignant tissues. In the first place, a greater motility characterizes the cancer

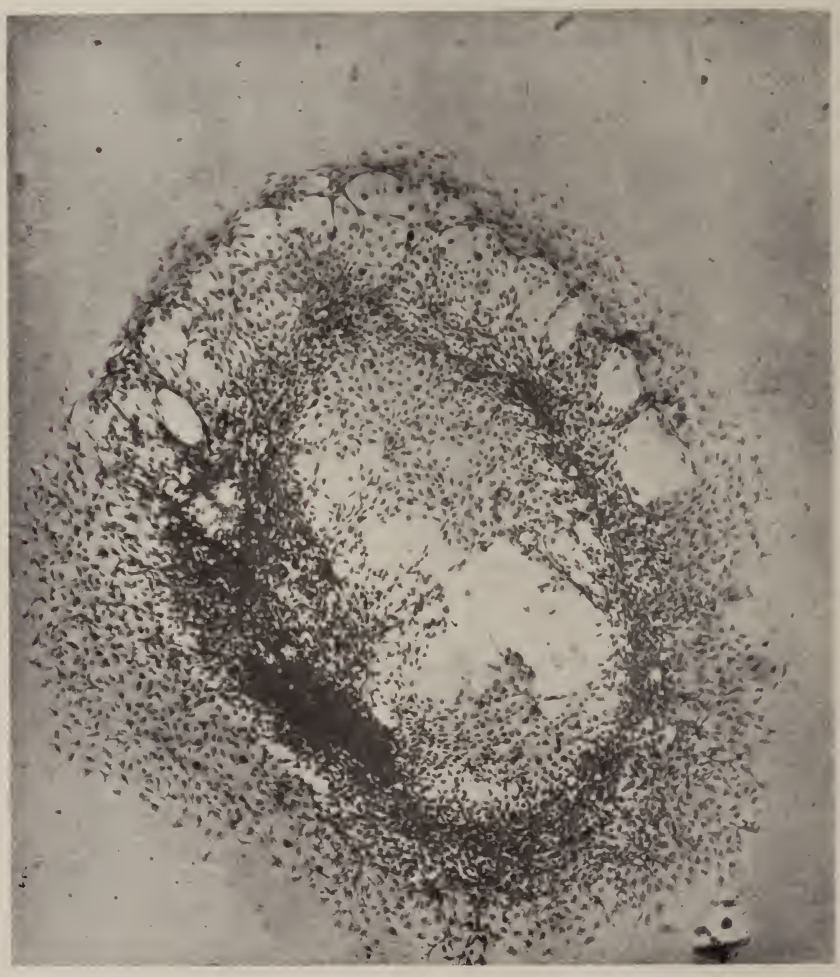

FIG. 2. Mouse sarcoma, ten-hour culture, showing extensive outwandering of cells which began within two hours after preparation was placed in incubator.

cell, especially the sarcoma cell, as compared with the corresponding normal element; a sarcoma cell may often be seen traveling through the medium at a rate almost equal to that of polymor-

phonuclear leukocytes (figs. 2 and 3). This fact, as has been pointed out before (22), probably throws light on the mechanism of the invasive growth and spread of cancer in the body. It is not necessary to regard the formation of metastatic tumor nodules as always a result of the passive transportation of cells from a primary tumor by the blood or lymph stream, when the cells may easily get from place to place by their own powers of locomotion. The writer has calculated that a tumor cell, if it survived the trip, might make its way from the middle of the breast to the axilla in less than four weeks.

A second important, and somewhat surprising difference between normal and tumor tissues lies in the fact that the continued propagation of certain normal cells, especially those of connective tissue, is, as a rule, much easier than in the case of tumor cells. Many carcinomata and sarcomata, especially those of human beings, will not grow even in primary cultures, while others, rat and mouse tumors, for example, grow actively for a few days but tend to die out even when transferred early to fresh plasma. On the other hand, it has been the writer's experience that connective tissue becomes very much more active in subcultures, so that after several transfers a most active cell multiplication is observed, dozens of mitotic figures being sometimes seen in a single preparation (fig. 4). The explanation for this difference in behavior is not obvious. It may be suggested either that some particular substance necessary for tumor growth is not being supplied in sufficient quantity in cultures or, stated in another way, that the cancer cell is really, in a sense, a highly differentiated cell which is particularly susceptible to changes in food supply and environment. That the cancer cell is a cell of lowered vitality is suggested by the results of some studies made by the writer on the comparative resistance of tumor cells and normal cells to heat (8). When normal connective tissue elements and cells from mouse or rat sarcomata were subjected to different degrees of temperature above that of the body, for variable periods, it was found that the latter were definitely more susceptible to heat than were normal actively growing connective tissue cells. Similar experiments with human tissues have

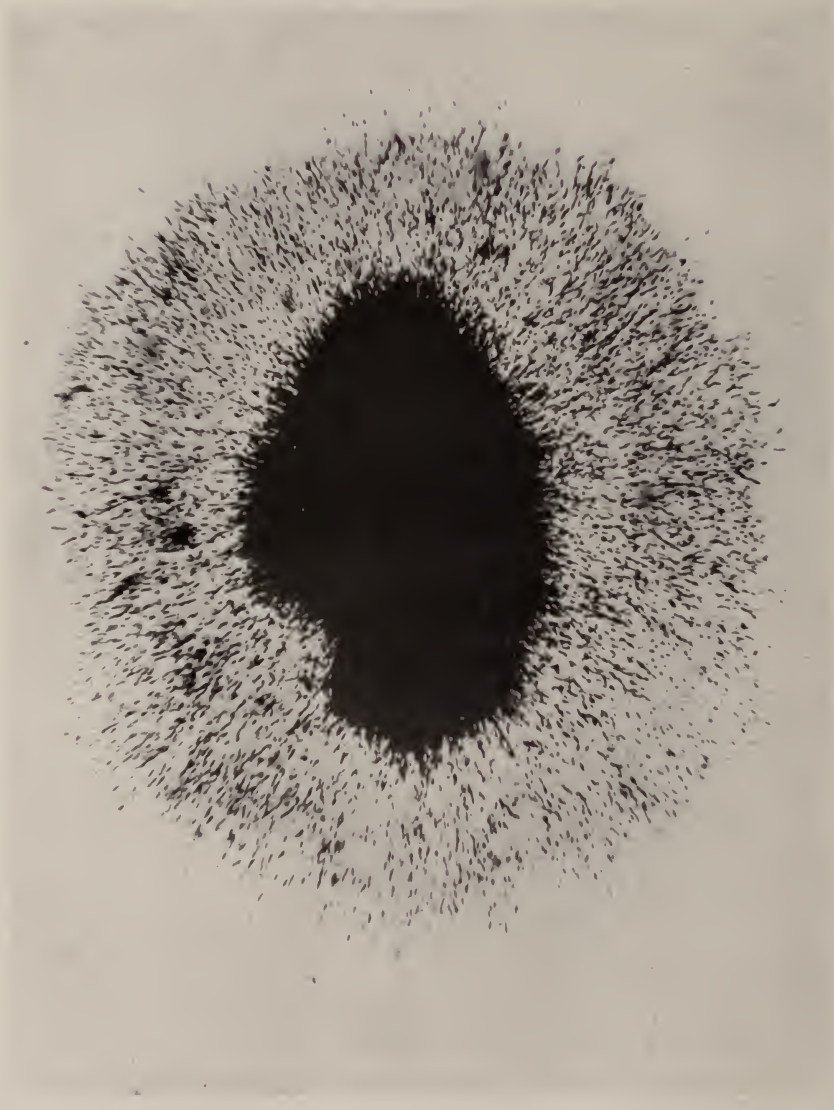

FIG. 3. Rat sarcoma, five-day culture, showing cells with numerous pseudopods creeping along the cover glass.

FIG. 4. Rat connective tissue cells, twenty-four days *in vitro*, showing very active multiplication after third transfer. Thirty mitotic figures were counted in the preparation. The tissue used was a piece of blood-vessel from an adult rat.

recently been attempted, in the course of which the writer has found that human connective tissue cells and wandering cells are quite as resistant to high temperatures as are those of the rat; a satisfactory comparison with human tumor tissue has not been made, however, on account of the difficulty experienced in obtaining sarcoma suitable for cultivation.

That the cancer cell is a particularly hardy cell, and that malignancy is due in some measure at least to this property, is a popular notion. This idea has, indeed, received some support from studies on the resistance of certain rat and mouse tumors to freezing and to toxic chemical substances (9, 10, 11). It is to be noted, however, that these studies, with one exception, were not comparative. The writer has found that many types of normal tissues isolated in tissue cultures are, like tumor cells, very resistant to low temperatures (12) and to many chemical agents. Ribbert (13) has called attention to the tendency of tumors to necrosis, maintaining that cancer cells are very susceptible to injuries of many kinds, and that this state of low vitality is referable to their poor vacularization.

While not accepting Ribbert's explanation, the writer is inclined, as the result of his own studies, to endorse the view that the cancer cell is a cell of relatively low resistance and that malignancy is to be attributed entirely to the capacity of the cells for unlimited growth.

## CANCER IMMUNITY

The results of the use of tissue cultures in the study of cancer immunity have been described fully in previous papers (14, 15, 16, 17) and need only be summarized here.

Although recent studies have shed new light on the question, the problem as to the exact nature of the resistance to transplantable cancer is still unsettled. Lambert and Hanes found that rat sarcoma cells will grow quite as well in the plasma of an immune rat (naturally or artificially immune) as in the plasma of a normal rat, an observation which affords further evidence that cancer immunity is not to be attributed to circulating antibodies of a cytotoxic nature.

It was shown further that the plasma from an animal of a foreign species (guinea-pig or rabbit) may serve also as a good medium for the cells. The fact that rat sarcoma cells can grow in such an alien plasma for at least thirty days would appear to invalidate Ehrlich's hypothesis of athreptic immunity.

On the other hand, it was discovered that if the guinea-pig or rabbit be first immunized against rat tissues by suitable injections of rat blood, skin, or tumor, the plasma of the treated animal becomes no longer suitable for the growth of rat cells, but is on the contrary distinctly toxic for any rat tissue. This experiment, besides showing that antibodies for tissue cells can be readily demonstrated in tissue cultures, proves also that cytotoxins are not specific for the cells used for immunization.

In further efforts toward demonstrating a cancer immunity reaction *in vitro* Dr. Edna Steinhardt and the writer attempted to cultivate rat sarcoma cells in immune rat plasma in association with normal tissues from immune animals (spleen, lymph-node, liver, and leukocyte emulsion), with the idea that the immunity phenomenon might be the result of the combined action of immune serum and some tissue or organ. We found that the growth of the tumor cells *in vitro* was not influenced by such a combination of factors. This negative result may have been due, however, to faulty technique, on account of which the essential protecting cells may have been injured, or present in insufficient number. In view of the recent work of Murphy (18) who attributes immunity against cancer to the action of certain lymphoid elements, it should be possible, by a proper combination of tumor cells and lymphocytes, to obtain an immunity reaction *in vitro*. Indeed it may even be suggested that the method of tissue cultures should serve to settle some of the questions which have been raised by this new work.

## STIMULATION OF GROWTH IN VITRO

This discussion of the application of tissue cultures to cancer problems would not be complete without reference to efforts directed toward the stimulation of normal and tumor cells, when

freed from body restraint. Without entering into a detailed discussion of physical and mechanical stimuli, the writer wishes again to emphasize the fact (19) that variations in the depth of the hanging drop and in the density of the fibrin meshwork (which can be altered by dilution) influence markedly the extent to which actively motile cells wander outward.

A single recent observation on a culture of human connective tissue exposed to a temperature of 45° for a few minutes may be of interest in this connection. Examination of the specimen just before exposure showed only two dividing cells in the entire preparation; ten minutes after heating, however, at least a dozen cells were seen in early stages of mitotic division. It may be that this single observation is without significance, but it does suggest further work on the effect of sudden elevation of temperature upon cell multiplication.

The action of various tissue extracts on growing cells has been studied by Dr. Carrel and his co-workers (20, 21), and very striking results have been reported on the effect of such preparations, especially those of chicken sarcoma, on embryonic chick tissues. The writer has not been able to verify their findings in such of the experiments as have been repeated, and is inclined to the opinion that the results of these authors are to be explained in other ways. It is certain that they do not possess any general application. An extract of human tumor, recently employed by the writer, appeared to inhibit rather than stimulate the growth of normal human cells.

Results following the use of certain chemicals (Scharlach R. and Sudan III) which have been shown to cause an abnormal proliferation of various tissues when injected into animals, have been disappointing. Cultures of chick embryo, and of rabbit skin and cornea, were treated with these dyes through several subcultures with no apparent effect, although they were applied in different ways and in varying strengths.

In spite of these negative results, this particular field seems to offer great possibilities. Since it is known that some sort of stimulus, or possibly the loss of some restraining influence does start cells within the body on a career of reckless multiplication,

it seems possible that patient work in this direction upon cells growing outside the organism may supply the key to this phase of the cancer problem.

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# THYROID TUMOR IN THE SEA BASS (SERRANUS)

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In January, 1911, in the course of autopsies on a few hundred fish dying spontaneously in the exhibition tanks at the Naples aquarium, the junior author obtained the five cases of thyroid enlargement in *Serranus* herein described. No other tumors were found in these examples of *Serranus*. In addition, many fish from the Neapolitan markets were examined, and among these a few neoplasms were found, but none involving the thyroid gland. He made sections of the tumors in *Serranus*, but was prevented by other duties from completing the study, and the State Institute became indebted to him for all the pathological material and data, including a sketch of the gross appearance of one of the cases. The material has been further worked over in the light of similar growths in other fishes and is here offered as a contribution to the pathology of fish tumors, particularly as they relate to those enlargements of the thyroid in fish which have of recent years become of some importance to research in the relationship of cancer and goiter.

The fish concerned are sea bass of the genus *Serranus*, belonging to a family of carnivorous fish, chiefly marine in distribution and inhabiting all the warmer oceans. The two species concerned, *scriba* and *cabrilla*, are marine forms common in the Mediterranean.

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[2] University of Würzburg.

## NORMAL THYROID IN SERRANUS

In order to examine the normal thyroid in *Serranus* and thus in part control our pathological material we have obtained from the Zoological Station at Naples specimens of both *S. scriba* and *S. cabrilla*. These were taken directly from the sea in the vicinity of Naples, probably early in 1915. We received only the heads of the fish. Of the nine specimens whose thyroids were sectioned, the heads measured 4.2 to 5.6 cm. from the tip of lower jaw to the free margin of opercle, save in one larger specimen, which was 8.3 cm. in length. The range of size was about that of the tumor fish. We have not minutely examined the anatomical structure of the thyroid region or the distribution of the thyroid throughout this region; these relations are apparently similar to those obtaining in salmonoid fishes (2), as is the structure of the normal thyroid epithelium itself, though in our sections the two groups are distinguishable histologically with respect to the latter. As in teleost fishes in general, the thyroid is not encapsulated. The follicles lie isolated and in groups throughout the connective tissues beneath the floor of the mouth.

*Serranus cabrilla* (fig. 1). The largest follicles have a diameter of about 0.4 mm. They vary widely in size, some being scarcely 20 microns across, while the larger are readily apparent under a ×6 hand lens. All are completely filled with perfectly uniform colloid which stains deeply; in section they are for the most part regular in shape—circular, slightly flattened, oval, elliptical, triangular, or occasionally polygonal; the elliptical outline, however, predominates. In no case are there any infoldings or outfoldings of the epithelium. The cells are far from filling the thyroid space available, and appear as groups or clusters here and there in the thyroid space, which is otherwise empty or contains connective tissue, capillaries, etc. Among the follicles themselves the extremely delicate connective tissue stroma itself is very scanty. In all the sections there is considerable free blood in the thyroid space.

Under a higher power, the epithelium is seen to vary greatly in height, showing all stages from squamous to high columnar,

though the predominating type is cubical or low columnar; the same follicle may show much diversity in the height of its cells. The nucleus is oblong in section, basal or central in position and may nearly fill the cell. The cells of the epithelium are not so definite as those of the more familiar salmonoid thyroid, and do not stain so precisely. In some follicles, the divisions between the cells are little evident and there appears a mere epithelial rim, faintly divided, about the colloid. It takes hemo-

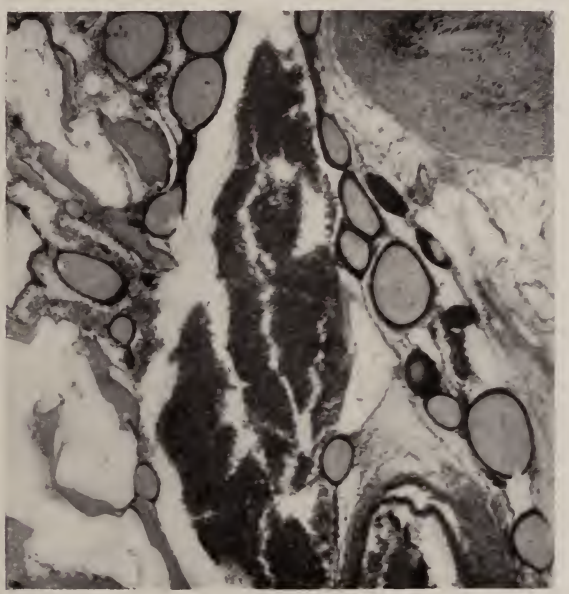

FIG. 1. Normal thyroid in *Serranus cabrilla*, in the vicinity of the central aorta. Free blood, bone, cartilage, etc. × 70.

toxylin with avidity, the color tending to spread over all the elements, while the cytoplasm is scarcely tinged with eosin. In the larger part of the follicles, vacuole-like bodies appear, which do not stain and which are evidently colloid secreted by the cell. Often the whole strip of epithelium has a pale border next the stained colloid of the follicle, due to the accumulation of these droplets of colloid in the luminal portions of the cell. The colloid may also nearly fill the cell, distorting the shape and position of the nucleus.

*Serranus scriba* (fig. 2). In the four specimens sectioned, the follicles are larger than those of *S. cabrilla*, the largest reaching nearly half a millimeter in diameter. Groups of two or three follicles are visible to the naked eye. The structure of the thyroid epithelium is substantially the same as that already described.

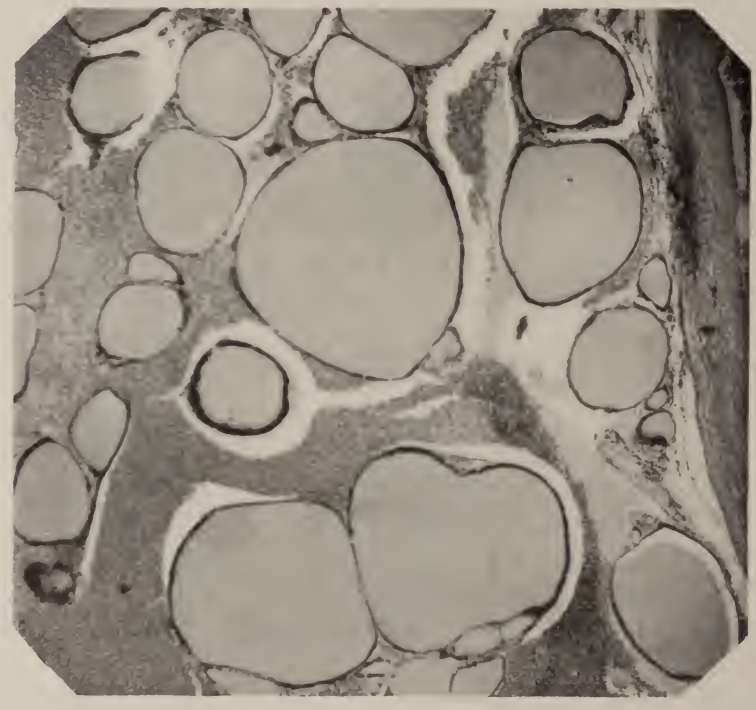

FIG. 2. Normal thyroid in *Serranus scriba*. × 70.

We have not sectioned large numbers of presumably normal thyroid of either species, and it is not to be inferred that the great difference in size of the follicles between the two species as represented by figures 1 and 2 is constant; an adequate series would probably show more uniformity between the two. But figure 2, while it suggests colloid goiter, and may represent somewhat enlarged follicles, shows a section of almost the whole of

one of the two or three heaps of thyroid units probably constituting the whole gland, which occupies only the smaller part of the thyroid space. It doubtless illustrates a condition fairly within the limits of physiological variation, limits which may, perhaps, be rather wide. On the other hand, the so-called colloid goiter of figure 3 not only filled the thyroid space but produced a macroscopic tumor.

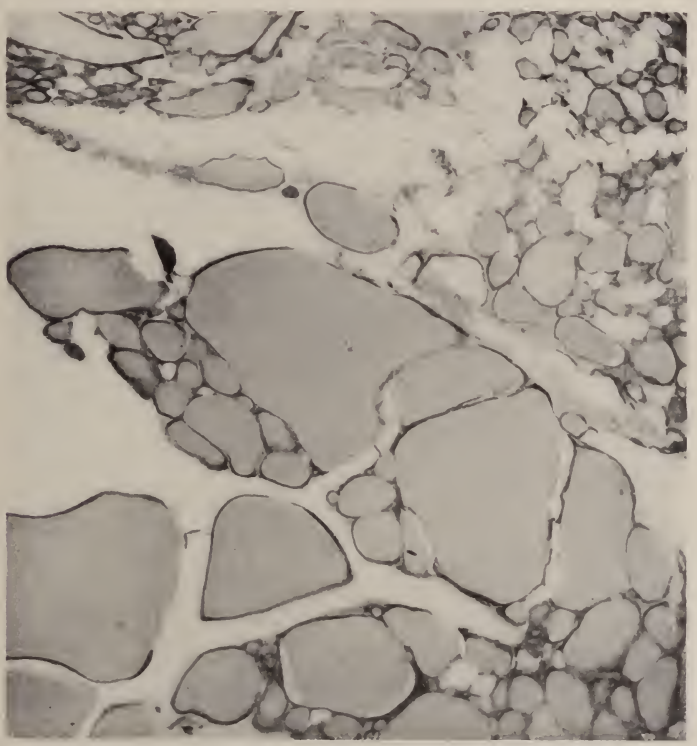

FIG. 3. Case I. Colloid goiter. × 70.

## THYROID TUMORS

The material consists of five tumors in as many fishes, though in one the enlargement was scarcely macroscopic.

Case I. Species uncertain; *S. cabrilla* or *scriba* (fig. 3). A small fish with a small nodule projecting on the right side near base

of first arch. The nodule and the growth in the thyroid region exhibit the same structure. The follicles are greatly increased in number, and many of them in size, and all are completely filled with colloid. The epithelium is scarcely elevated above normal, and is nowhere infolded to any extent. Despite slight areas of thyroid overgrowth, these sections represent nothing more than colloid goiter.

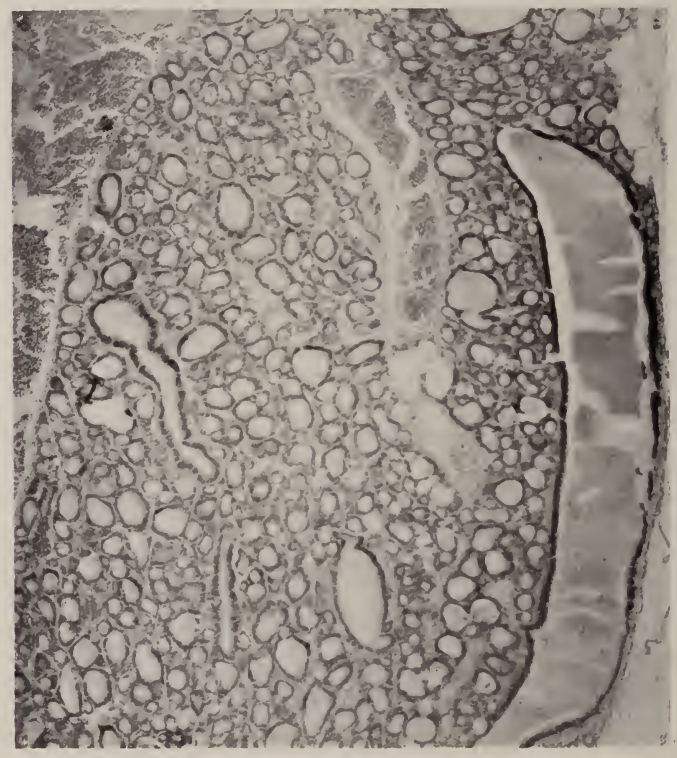

Fig. 4. Case II. Thyroid tumor. Beginning cyst formation. × 70.

Case II. *S. scriba* (fig. 4). One of the larger fishes of the series, with two small lobes of thyroid growth, one on each side of throat.

The growth within the thyroid region is entirely and uniformly alveolar. Colloid is everywhere present, and the epithelium is

abundant and distinctly elevated. The process in this fish is intermediate between the succeeding cases and colloid goiter, differing from colloid goiter in the smaller size of the follicles and the prominence of the epithelium, and from all the remaining cases in the abundance of colloid and the less advanced exhibit of epithelial growth.

The macroscopic lobes show the same general structure, with the beginning of cyst formation. The epithelium of the cysts is very much higher than that of the non-cystic portions, and its cells are actively secreting colloid, which shows as a pale border along the luminal edge of the row of cells. The section cutting a flattened cyst gives rise to a striking simulation of ducts. Much of the lobular growth has taken on the tubular type, due to the arrangement of cells in rows.

Case III. *S. scriba*. This example showed no external enlargement, though on medisection of the floor of the mouth the thyroid region was found completely filled with the thyroid growth. Microscopically, the structure is not at all cystic, but shows in contrast to the foregoing specimen a minute alveolar arrangement. All the thyroid units have remained small and there is no trace of papillary development or of cyst formation; colloid is little in evidence but is present in the larger alveoli. The epithelium varies from cubical to columnar. In some portions of the growth the alveoli are unusually small and without lumina, giving a more solid appearance to the structure, while in others an increase in the stroma affords an unusual picture in thyroid tumors. Several instances of small hyaline blood-vessels with obliterated lumina occur.

Case IV. *S. scriba* (figs. 5 and 6). This, the largest tumor, was found in the largest fish of the series. Figure 5 is a sketch about natural size, made by Dr. Vonwiller at autopsy. The tumor had pushed out from the thyroid region chiefly toward the ventral aspect of the throat, where it appeared on either side of the isthmus as outstanding elongate lobes the largest of which was 25 mm. long. The floor of the mouth was distinctly raised by the growth at the third pair of arches but the tumor had not vegetated free in the mouth cavity.

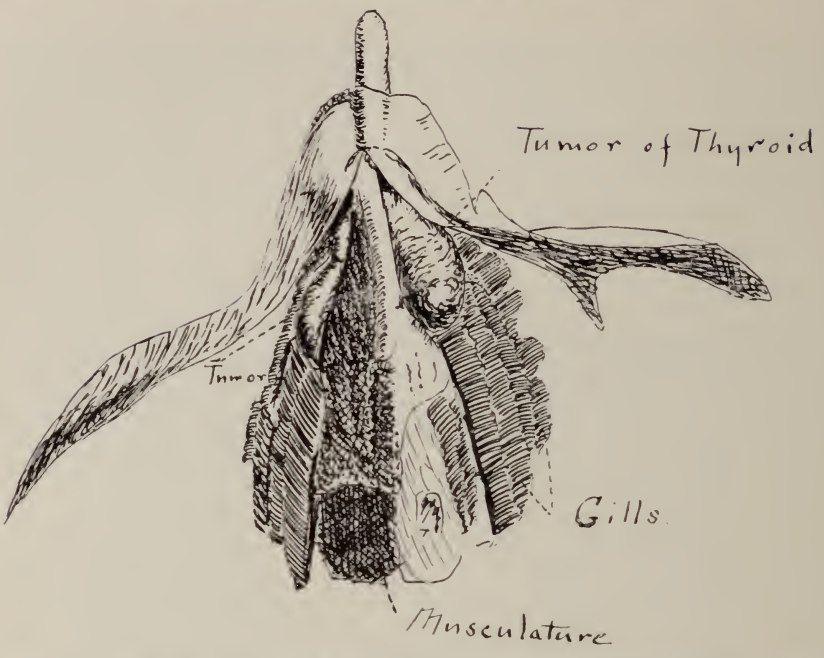


FIG. 5. Case IV. Field sketch by Dr. Vonwiller, of ventral aspect of head of *Serranus scriba* with thyroid tumor. Externally visible lobes, one partly cut away. × 1.

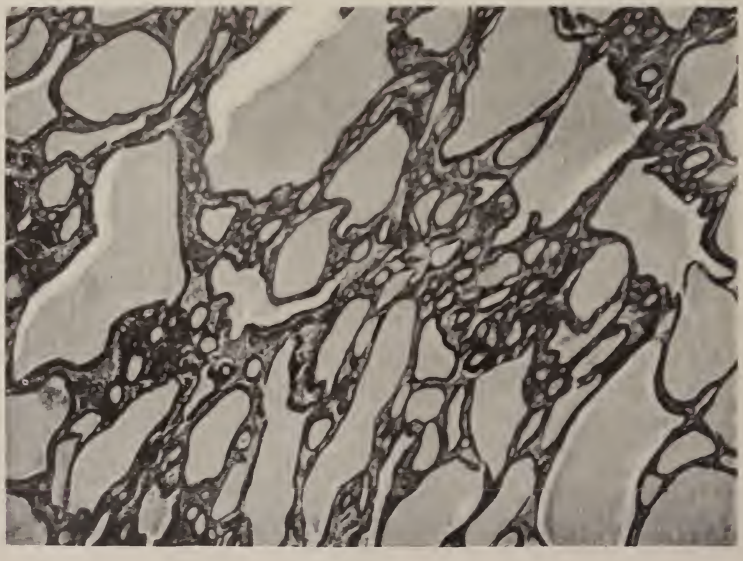

FIG. 6. Case IV. Thyroid tumor. × 70.

On gross section the tumor was found to be very cystic, the cysts being filled with a solidified transparent gelatinous mass, the colloid of the thyroid gland. The tumor occupied the whole thyroid region, from which its lobes vegetated chiefly toward the ventral aspect of the throat.

Sections through the thyroid region itself show under a low power an alveolar structure as the prevailing type of growth; many of the alveoli are very irregular, and some are sufficiently infolded to suggest the papillary type, while a few have enlarged and produced distinctly cystic areas. Colloid abounds throughout the section. The epithelium varies greatly in height; occasionally cubical in portions of alveoli, and scarcely higher than normal, by far the greater proportion is columnar and becomes very high in many places throughout the overgrowth. No normal follicles are anywhere to be seen. The cells of much of the epithelium are turgid, closely massed, and do not stain precisely but take the hematoxylin diffusely; there is no sharp distinction between nucleus and cell body, nor even between individual cells. While the growth is characterized as alveolar, small areas of solidly packed cells are to be found; in these the cells have often still further lost their definite outlines and present a nearly uniform mass diffusely colored with the nuclear stain.

The submucosa is infiltrated here and there with offshoots of the tumor mass and some of the sections show a slight but distinct invasion of the outer layers of the aorta, but the growth has no pronounced infiltrative character; nodules are found in bone spaces, it is true, yet clear evidence of destructive infiltration of bone or cartilage is lacking. Small arteries with thickened walls and evidence of calcification are not infrequent.

The tumor lobes which appear externally (fig. 5) have substantially the above structure, though they are much more cystic. The red cells of the blood have found their way into some of the alveoli and, with greatly swollen and vesicular cytoplasm, are in a state of degeneration.

Case V. Species uncertain, *S. cabrilla or scriba* (fig. 7). The fish bearing this tumor was one of the largest of the series, the floor of the mouth measuring 3.5 cm. from the tip of the tongue

to the level of the fourth arch. On the left side of the throat, a hemispherical thyroid nodule 6 mm. in diameter was the only external evidence of abnormal growth.

Upon microscopic examination, the growth proved to be of the same character as Case III. The structure is looser however and the epithelial growth is far from filling the available space. The latter is in many places separated and broken up by a very cellular stroma rich in capillaries, which is in places more abun-

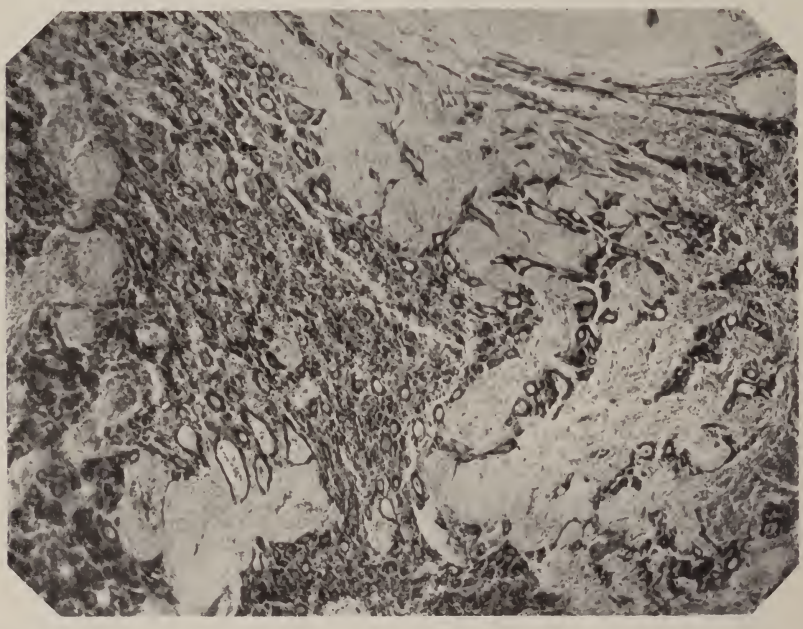

Fig. 7. Case V. Thyroid tumor. Fibrosis and regression. × 70.

dant than the epithelial growth. Fields of connective tissue overgrowth occur and the alveoli are shrunken from pressure atrophy and often drawn out into long processes and strands. The whole picture indicates a process of regression. The structure of the external lobe is quite the same as that of the tissues in the thyroid region.

Cases III, IV, and V all show peculiar degenerative changes in some of the small vessels and the red cells, which we have not

hitherto seen in thyroid growths of fishes. Many minute vessels are packed with greatly enlarged red corpuscles which have become irregular in shape, with greatly diminished and eccentric nuclei. They do not stain normally, the cytoplasm showing a brownish yellow with hematoxylin and eosin. The vessels, in a further stage, may undergo fibrosis until the lumen is filled, when they resemble the connective tissue tubercles which represent a reaction to parasitic worms such as nematodes, in the tissues. We were unable, however, to find any definite evidence of such parasites. These vessels finally become completely hyaline.

Larger, solid, hyaline nodules occur with little evidence of tissue structure, some of which show traces of elastica with an orcein stain; they are probably blood-vessels, and perhaps a phase of the process described above. Since it does not appear that these structures have any essential relation to the tumors, they need not be further considered at this point.

## DISCUSSION

Thyroid tumors have long been known to occur in fish, but until recently scarcely outside the salmonoid group, and only rarely save among domesticated fish, where they are endemic and widespread. An extended study of this subject with numerous citations of the literature has been made by Gaylord and Marsh (2). A recent observation (3) extends the range of this disease in fish culture to India, and its geographical distribution appears to be as wide as is the domestication of the salmon family. On the other hand, its occurrence among wild fish of this group is comparatively rare and in this country comprises, as far as is known to us, three examples, in a trout, a whitefish, and a landlocked salmon, representing respectively the genera *Salvelinus, Coregonus, and Salmo*. All these instances of thyroid enlargement were acquired in fresh water.

In marine waters thyroid tumors are apparently of still rarer occurrence. The first to be recorded, and the only one known to us besides the present specimens, is the case described by Cameron and Vincent (1). This was in the "dogfish" of the

Pacific coast of North America, a small shark of the genus *Squalus*. Its thyroid, which was encapsulated, showed an enlargement ($\times$ 3.5), though relatively slight when compared to the growths in our specimens or to those common in trout. The authors liken this growth to adenomata with local areas showing distinct infiltration and others of sarcomatous appearance, and quote the opinion of Adami, to whom they had submitted sections, and who commented upon the resemblance to true carcinoma.

In conjunction with the normal thyroids of the corresponding species from the same region, which permit of direct comparison and afford a satisfactory control, our tumors, though few in number, form a fairly well graded series. The thyroids of the controls do not exhibit as much variation as those of trout which we have seen from natural waters. They were taken in the winter and possibly represent a simpler structure corresponding to a reduced physiological activity due to the season. The gland is not encapsulated, the follicles are scanty in numbers, quite simple and regular, uniformly distended with colloid, and without any definite hyperplasia. Among the tumor fish, one specimen is plainly colloid goiter, the next is microscopically a step in advance toward epithelial increase of large alveolar type and shrinkage of the colloid content. In the others, the epithelial overgrowth is predominant over the colloid, the cells have become high columnar, and the growth has taken on a definite adenomatous structure. Finally, marked regression is indicated in one specimen.

We do not regard the growth in these specimens of *Serranus* as giving pronounced evidence of malignancy. The submucosa has been freely entered and the aortic wall and bone definitely penetrated in a few instances, but little infiltration is exhibited throughout the growths as a whole. In this respect they do not approach the process as exemplified in the fresh water salmonoids or in the case of *Squalus* referred to above. The bodies of the fish were not examined for metastases. We believe the tumors to be true neoplasms representing early stages of processes which are of essentially the same nature as the other thyroid overgrowths in fish which have been the subject of investigation. The proc-

ess is probably a single entity from its earliest stages, and the labeling of the various pictures presented is not a matter of particular importance. Microscopically one recognizes colloid goiter, areas of adenoma, cystadenoma, and adenocarcinoma.

As is well known, iodine acting through the water medium, even in great dilution, has a marked action upon thyroid hyperplasia or more advanced overgrowth in fresh water fish, reducing the heightened epithelium, restoring colloid, and effecting regressive changes in general. The feeding of marine fish has a similar effect, and such a diet has been urged as an expedient for the prevention of thyroid overgrowth in hatcheries, the empiric results sufficiently justifying such a measure. The mere finding of thyroid tumors in marine fishes which have never left sea-water is therefore a matter of much interest. Our specimens become of additional importance from the fact that they were removed from the sea to an aquarium supplied with sea-water, and there habitually fed with marine fish, chiefly the pilchard (*Clupea pilchardus*). They received no mammalian tissues. Unfortunately, the duration of the sojourn of each fish in the aquarium can not be stated, nor whether they had abnormal thyroids when captured from the sea. The presumption is that they had remained in captivity at least for a number of weeks, and possibly for months. At any rate, they acquired the growths in sea-water, which contains a far greater concentration of iodine than that which is effective in reducing thyroid hyperplasia in fresh water. It is, however, not necessarily excluded that the use of marine fish as food was responsible for the regression indicated in Case V. The others (Cases II, III and IV) may possibly have been introduced into the aquarium but a short time before their death, and might have soon shown regression, had they lived. Case I is somewhat anomalous, since most fish subject to thyroid enlargement show no such condition at any stage of the growth. An interesting comparison may here be made with the introduced European sea trout (*Salmo trutta Linnaeus*) known in this country as the Scotch sea trout. This salmonoid, so far as experience goes, does not develop macroscopic thyroid tumors, though bred in fresh water side by side with

those species most subject to them; it exhibits, however, occasional examples of microscopic colloid goiter under these conditions. In Europe, this fish is migratory, frequenting alternately marine and fresh water. Possibly in both cases the thyroid abnormality represents a process distinct from, and unrelated to, the thyroid growths known in other fishes.

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# FURTHER OBSERVATIONS ON SO-CALLED CARCINOMA OF THE THYROID IN FISH

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Our knowledge of the neoplasms in fish has not been materially augmented since the articles of Marianne Plehn in 1906, 1910, and 1912. It is well known that several types of tumor are found in the teleosts, but for the purposes of cancer research, descriptions of new growths in the lower animals serve only to extend our knowledge and to confirm our impressions regarding the widespread distribution of neoplastic processes throughout the animal kingdom. Only when such tumors have been made the subject of experiment or of more extended study, is their significance enhanced. In fish, the particular type of neoplasm which has been so studied is the so-called carcinoma of the thyroid in the salmonoids. Just as in other biological groups a certain type of neoplasm preponderates, as in mice carcinoma of the breast, and in rats and chickens the sarcomas, so the salmonoids present that type of neoplastic process which even in mammals bears a close relation to the character of the water supply. From this standpoint it is not surprising to find carcinoma of the thyroid in fish.

The disease was first described by Bonnet in 1883. Although this author did not recognize its nature, Scott, in 1891, regarded it as cancer, and Plehn, in 1902, recognized it as a neoplasm of the thyroid gland. In the same year it was described by Gilruth as epithelioma affecting the branchial arches. A comprehensive histological study based on some ten specimens of fish was made by Pick in 1905. Our own contributions to this subject date from 1908, with the description of conditions existing in a hatchery in which hundreds of fish were affected. The disease has

been studied also by Marine and Lenhart, beginning in 1909; these authors consider it to be endemic goiter. Our studies, covering a period of six years, were published in monograph form in 1914, and led to the following conclusions regarding the nature, distribution, and significance of the disease.

1. The disease known as gill disease, thyroid tumor, endemic goiter, or carcinoma of the thyroid in the *Salmonidae*, is a malignant neoplasm.

2. The disease occurs in fish living under conditions of freedom in populated areas.

3. When introduced into fish-breeding establishments it becomes endemic, with occasional epidemic outbreaks.

4. Normal fish taken from the wilderness may be made to acquire the disease when placed in fish-breeding establishments where the disease is endemic.

5. The feeding of uncooked animal proteid favors, and the feeding of cooked animal proteid retards the disease as compared with the uncooked. Feeding alone is not an efficient cause. It must be combined with an agent transmitted probably through the water or food, or both.

6. By scraping the inner surface of water-soaked wooden troughs in which the disease is endemic, an agent may be secured which, from its action upon the mammalian thyroid when administered through drinking water is no doubt the cause of the disease in the fish confined in these troughs.

7. The agent is destroyed by boiling.

8. Fish in all stages of the disease are favorably affected in the direction of cure by the addition to the water supply in suitable concentration of mercury, arsenic or iodine.

9. The effect of mercury, arsenic and iodine in carcinoma of the thyroid in fish, and the subsequent positive experiments with metals in mammalian cancer, are probably the expression of a therapeutic relation of these elements to carcinoma.

10. Certain species of the *Salmonidae* have an almost complete natural resistance to the disease.

11. Certain lots of fish of susceptible species show a high degree of immunity to the disease.

12. Spontaneous recovery occurs in a considerable percentage of individuals.

13. Removal from ponds in which the disease is endemic to natural conditions, or a change to more natural food, increases the percentage of spontaneous recoveries.

14. Spontaneous recovery appears to confer a degree of immunity against recurrence.

15. The percentage of spontaneous recoveries in the early stages of the disease appears to be higher than in the later stages of the disease.

16. The incidence of the disease increases with the age of the fish, at least up to five years.

17. Thyroid enlargement, and changes presenting at the end of five months a picture of diffuse parenchymatous goiter, were induced in mammals by giving them water to drink in which had been suspended scrapings from troughs in which the disease is endemic. Control animals which received the same water boiled failed to develop thyroid changes. That these enlargements and changes are the first stages in mammals of the same disease which occurs in fish inhabiting the troughs from which the scrapings were obtained, is an inference which we believe further experiments will justify.

18. The disease is endemic in a very high percentage of all trout hatcheries in the United States.

19. The occurrence of the disease in wild fish, its introduction into fish-cultural stations, its localization in certain troughs or water supplies, the method of its spread, its transmission to mammals, the efficacy of three well-known inorganic germicides in the treatment of the disease, the destruction of the agent by boiling, and the phenomena of spontaneous recovery and immunity, strongly indicate that the agent causing the disease is a living organism.

20. No evidence has yet been produced to indicate the direct transmission of the disease from individual to individual.

21. In many of its phases the disease is identical with endemic goiter. As there is no line of demarcation between what is called endemic goiter and what we believe we have clearly shown

to be cancer of the thyroid, we hold that endemic goiter and carcinoma of the thyroid in the *Salmonidae* are the same disease.

Since the publication of these conclusions, certain new facts have been disclosed which serve to extend or strengthen some of our conclusions in regard thereto. That the disease is widespread geographically is indicated by reports which we have received of its occurrence in India. T. Southwell, Director of Fisheries for Bengal, Bihar, and Orissa, wrote us regarding it in 1914, and later sent us specimens of rainbow trout taken in the vicinity of Naini Tal, with visible thyroid tumors. These proved on microscopic examination to be thyroid carcinomata of the usual type. Facts pertaining to this observation have since been published by Southwell, with two excellent illustrations, in the records of the Indian Museum.[1]

In the literature of the subject there is but one reference to the occurrence of visible thyroid tumor in salt water fish, and this only in *Salmo salar Linnaeus*, the Atlantic salmon. But as these fish at certain times ascend fresh water streams, they are not strictly salt water fish. It is, therefore, of great interest to learn that four cases have been found in two species of sea bass (*Serranus*). These fish were discovered in the Naples aquarium by Dr. Paul Vonwiller and will be later described in detail by Marsh and Vonwiller. The growths are, in general, like those of the *Salmonidae*, but are not so advanced. Colloid is found in all sections of the tumor. The epithelium presents all stages from low cubical to high columnar, and is not greatly infolded, but presents the appearance of adeno-carcinoma. In *Serranus* there is very little evidence of infiltration and no metastases were demonstrable, as only the heads of the fish were available for investigation. These fish were always in sea-water, and they were fed exclusively on a diet of marine fish, which is said to prevent thyroid over-growth. This is the first observed instance of thyroid tumor in salt water fish outside the *Salmonidae*.

[1] In Records of the Indian Museum (Calcutta, 1915), vol. xi, p. 317, Southwell implied that these trout were taken in open natural waters. In a personal communication he has since informed us that this was an error and that those specimens bearing thyroid tumors came from a hatchery.

Still more striking is the discovery by Cameron and Vincent, of a thyroid tumor in an elasmobranch, *Squalus*. *Squalus* is a dogfish or small shark. In the examination of 217 specimens of dogfish from the Pacific coast, these authors found one in which the thyroid was enlarged to about three times the usual size, and was pear-shaped and nodular, instead of leaf-like and flat as it normally is. The enlarged gland contained macroscopic cysts, while cyst-like dilatations of the alveoli with cell proliferations projecting into them were visible under the microscope. There were areas of normal vesicles containing colloid, but the alveoli in the more characteristic portions of the gland were filled with solid masses of cells; in some places, all alveolar structure was lost, the growth being made up of irregular masses of cells. The general appearance of the growth was adenomatous, but in certain regions infiltration of the interstitial tissue was marked. The picture described by these authors is characteristic of the disease in the *Salmonidae*.

The point of special interest is that the thyroid in *Squalus* is an encapsulated gland. Besides this one example of gross enlargement of the thyroid in *Squalus* these authors describe two specimens in a group of twelve subsequently examined, which showed distinct pathological changes on microscopic examination. In these, large portions of the gland were devoid of colloid, the vesicles being entirely filled with epithelial overgrowth. The sea-water of the Strait of Georgia, where the *Squalus* was obtained, is about two-thirds normal ocean salinity, and doubtless two-thirds normal marine iodine content; this however is much above the concentration of iodine which in fresh water reduces thyroid hyperplasia.

These observations, together with the evidence which has been collected regarding the existence of thyroid carcinoma in fish living under natural conditions, and hence unaffected by overfeeding, over-crowding, etc., strengthen the conclusion that the disease is not the result of artificial propagation. The occurrence of the disease in sea fish bears rather strongly upon that theory of thyroid hyperplasia which attributes it to a deficiency of iodine in the food or environment. In fact, so far as fish are

concerned, the similar results obtained with mercury and arsenic would seem to show conclusively that iodine acts in a way similar to the metals, and not by supplying a deficiency of iodine in the gland. The deduction that the feeding of vegetable food, cooked protein, or chopped sea-fish, or of so-called natural food such as maggots, bugs, etc., retards the progress of the disease in hatcheries, and that the administration of uncooked animal protein is a contributing factor rather than the cause of the disease, has been strengthened by the observation of the lesion in trout fed on cooked vegetable food. These fish came from a commercial trout hatchery in Pennsylvania. The first food on which they were fed as fry, was beef liver for about two months; then a mush made from wheat flour was added, and gradually increased in relative proportion until, during the next four months, all liver had been eliminated; subsequently only cooked flour mush was employed. Trout kept on such a diet grow slowly, but mature and breed, and are sold for table use at what is probably a record price for commercial trout. They nevertheless acquire thyroid tumors, which appear in both yearlings and older fish; the growths are small, however, and only a small percentage of the fish are affected. Microscopically, most of the lesions typical of carcinoma in the *Salmonidae* are seen. This is cancer in fish. The occurrence of the disease in *Serranus*, sea-fish fed exclusively upon marine fish food, also strengthens the conclusion that feeding is not the essential cause of the disease.

The observation that mercury, arsenic and iodine, when introduced even in small amounts into the water containing fish with thyroid tumors, bring about a marked diminution in the size of the neoplasm, develops increased significance in the light of recent experiments reported by McCarrison. This author has studied the prevalance of goiter in the Lawrence Military Asylum for children at Sanawar. In this institution, located in the lower ranges of the Himalayas at an elevation of about 6000 feet, there are 500 European and Anglo-Indian children (of both sexes); two-thirds of them are of pure European descent, the remaining one-third being of mixed parentage. A careful study of the records, which went back for some fifteen years, showed

that the incidence of goiter in this asylum, with the exception of a few years, was about 20 per cent of the inmates. McCarrison finally traced the source of the endemic to the drinking water, which was in part rain water and in part spring water; both sources of supply were open to fecal contamination and showed abnormally high bacterial content. Beginning April 19, 1913, McCarrison undertook the experiment of sterilizing the water by chemical means in the boys' school and barracks, while for control purposes the water supply of the girls' school and barracks remained as before. At the commencement of the experiment, there were 284 boys in the school, of whom 57 were goitrous. There were 216 girls in the school, of whom 57 were goitrous. On the 25th of November, 1913, when the experiment was completed, the number of cases among the boys had been reduced to 27, whereas among the girls there was an actual increase of cases above the 57 first recorded. The method employed was the partial sterilization of the water by the use of iodine in the form of potassium iodide and iodate, the proportion of iodine being one part in 70,000. This method was used until July 2, 1913, midway in the experiment; from July 3 on, chlorine was largely substituted for iodine, with the continuation of the results observed in the first instance; in the latter part of the experiment the amount of iodine was reduced to one in 911,000 parts. This is still five times as great a concentration of iodine as we used in treating goiter in fish. In discussing the question whether the iodine acted in this case as a purifier of the water, especially as a germicide, or whether its effect was to be attributed to its supposed physiological influence upon the thyroid gland, McCarrison stated that he had obtained equally favorable results in the treatment of goiter with thymol; hence he was not inclined to attribute to iodine a distinctly physiological action in the treatment of goiter.

McCarrison's experiments do not show conclusively that the iodine worked by destroying an agent in the water supply, for the possibility still remains that it may have exerted some germicidal effect in the individual drinking the water. It is to be regretted, therefore, that he did not entirely substitute chlorina-

tion of the water for treatment with iodine. As a confirmation of the results obtained with iodine by Marine and by ourselves, however, his work indicates a very valuable application of this method in the practical treatment of goiter in human beings.

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# THE EFFECTS OF CANCER TISSUE AND OF NORMAL EPITHELIUM ON THE VITALITY OF PROTOZOA

## DIDINIUM NASUTUM. II

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The investigation of the cause of cancer resolves itself into a search for the causes of abnormal cell growth and cell division, and is essentially a cytological or biological problem. Numerous theories have been advanced, each successive one more or less plausible than the others, but all with a weak link somewhere in the chain of evidence. These hypotheses are familiar to every student of cancer problems and need not be reviewed again in the present paper, in which I wish to summarize the results of experiments with cancer and normal tissue on a free living ciliated protozoon—*Didinium nasutum*.

The main problem in this line of cancer work may be approached by way of a secondary problem which may be stated in the form of a question, as follows: Is there anything in cancer tissue, different from anything in normal epithelium, that has a specific effect on normal free-living cells?

Juices and extracts of malignant tumors have frequently been injected into normal tissues and cavities, in order to determine whether they would call out a specific response in the form of growth and division of the normal tissue cells. Apart from the usual connective tissue reaction these experiments have not led to definite conclusions, due perhaps to the fact that they are performed on highly complex animals in which the powers of protection, of control and regulation, are well developed, so that a local reaction to the injection is prevented by the activities of the organism as a whole. If the dose is large enough the animal is

seriously sickened, or dies. No local effect on the epithelial cells in the vicinity of the injection is produced, possibly owing to the difficulty in obtaining a local action, nor is there any way of measuring the effect of such injections on the vitality of normal epithelial cells. It is for experiments directed to this object that the protozoa are particularly helpful. Here we have a normal free-living cell, and the effect of cancer extractives on its vitality can be ascertained and measured without interference by regulative physiological responses of an organism as a whole. Protozoa used in this way are termed "indicators."

Woodruff and Underhill (1913) were the first, so far as I know, to use protozoa as living indicators in cancer work. They used *Paramecium aurelia* for the normal cell and human breast carcinoma for the cancer material. They obtained some very interesting and significant results as follows: first, that undiluted cancer material in the form of an extract, produces a well-marked depressant effect on the vitality of *Paramecium*, in some cases leading to death of the indicator in two or three days; second, that weaker or diluted extracts of the cancer material may or may not produce a definite stimulating effect. The comparisons were made only between cancer tissue and normal epithelium; the relative vitality of the *Paramecium* experimented on compared with that of the normal free-living *Paramecium* at the same time was not given. If cancer extracts are capable of producing either a depressant or lethal effect, or a stimulating effect, on protozoan vitality the question naturally arises: does normal epithelial tissue extract have similar effects on *Paramecium?*

These results of Woodruff and Underhill were so suggestive that I thought it worth while to repeat the experiments using some precautions not considered by them. They used five different specimens of mammary carcinoma, which gave somewhat varying results, due possibly to the varying degree of necrosis of the tumor material employed in getting the extracts. These were prepared by mincing portions of the freshly excised cancer in a small chopping machine, adding water to five times the weight of the tumor material used, and then heating the mixture

slowly to the boiling point. The extracts thus prepared were used in full strength doses and in various degrees of dilution, with results as given above. The liquid was filtered and the *Paramecium* to be tested was isolated in 5 drops of the extract thus prepared.

One objection to the use of human cancer material is the extreme difficulty of getting rapidly growing non-necrotic tissue and the practical impossibility of getting tumors of identical age. For this reason I have in the following experiments employed a rapidly growing mouse carcinoma, with a high percentage of takes. This was inoculated into batches of mice at such times as to give fresh mouse tumors of approximately the same age and size every day during the period of the experiment (15 days). The use of heat, employed by Woodruff and Underhill, was avoided.

An objection to the use of *Paramecium* as an indicator is that *Paramecium* feeds upon bacteria, and the quantity and quality of its food cannot well be measured. A reduced division rate may be due to an inadequate or an improper food supply, or it may be due to loss of appetite caused by chemical substances in the medium, or to some disturbance in metabolism due to the conditions of the experiment. On the other hand, an increased division rate may be due to effects of chemical substances on the quantity and quality of the food supply as well as due to stimulation of the vital activities of *Paramecium*. Furthermore, the bacteria are sources of putrefactive changes which cannot be measured. For these reasons I have employed as indicator a free-living ciliated protozoon which lives on other protozoa and will not eat bacteria. There are several such forms to choose from: *Actinobolus radians* eats only *Halteria; Spathidium spathula* eats *Colpidium; Didinium nasutum* eats large ciliates like *Paramecium*. Of these forms *Didinium* was finally chosen for the experiments because of its feeding habits and its large size.

## A. MATERIALS AND METHODS

### *1. Didinium nasutum*

This organism, used as indicator in these experiments, is a fairly common protozoon of fresh water ponds. It is one of the predatory ciliates belonging to the order *Holotrichida* of the *Infusoria*, and feeds by capturing and swallowing other *Infusoria*. Among such forms serving as food, the common "slipper animalcule"-*Paramecium*, is greedily seized and rapidly digested. As *Paramecium* may be easily cultivated in large numbers in standard hay infusion, I have used it throughout the experiments as the standard diet of *Didinium*.

A few preliminary observations showed that a single isolated *Didinium* in good health, together with its progeny, can easily take care of nine individuals of *Paramecium caudatum* in 24 hours, and this number of *Paramecium* was therefore adopted as the standard daily ration.

The cultural methods employed were the same as those commonly used in protozoon life history work. A single individual was isolated on October 28 from Van Cortlandt pond water and placed in a ground glass culture dish containing $\frac{1}{2}$ cc. of Great Bear spring water. The *Paramecium caudatum* to be used as food were counted out and placed in the spring water with it. The culture dish was then covered and set aside in a glass moist chamber. Twenty-four hours later the culture dish was examined and found to contain eight individuals of *Didinium* and no *Paramecium*. These eight individuals from the original one furnished part of the material used in the subsequent experiments, forming what I call the A-series. Five of the eight were isolated in culture dishes and each supplied with nine *Paramecium caudatum*, while the remainder were set aside with food as "stock," all being kept in the same moist chamber. This procedure was followed out daily, one from each of the five lines being isolated and fed daily with a constant number of *Paramecium*.

In the same way a second set of five lines of *Didinium*, known here as the B-series, was started at the same time and maintained in the same way, the two series giving all the control

material for the experimental work and furnishing all the individuals used in the tissue series.

*Didinium* reproduces exclusively by simple division, and the division rate per day has long been recognized as a suitable index of infusorian vitality. When conditions of the environment are poor, this organism, like many other protozoa, encysts, or secretes about itself a thick and resistant membrane within which it lies dormant, often for long periods of time. When conditions are again suitable it emerges from the cyst and resumes its normal vegetative activities. *Didinium* may also encyst even when the environmental conditions are suitable for continued life. Encystment at such times is due to physiological conditions of unknown nature, and is accompanied by cytological phenomena involving nuclear changes similar to those which the organism undergoes during the process of conjugation. It is a process of parthenogenesis resulting in complete nuclear and cytoplasmic reorganization, after which there is renewed vitality manifested by greater activity in movement, feeding, and division when the organism leaves the cyst. *Didinium* also conjugates; two individuals fuse in the region of the mouth and remain together from eighteen to twenty-four hours, or during the interchange of gametic nuclei, when they separate. This process, however, is prevented in culture work by the daily isolation, and need not be touched upon in the present paper. Natural encystment with parthenogenesis, however, occurred twice during the period covered by the experiments from October to May, and these processes, with the normal life history of *Didinium*, are described in the *Journal of Experimental Zoology*, vol. xix, 1915.

Records were kept, not only of the daily division rate, but also of the number of *Paramecium* eaten during the 24 hours, the number of deaths and the number of encystments. The number of *Paramecium* was determined by subtracting the number found alive at the end of the observational period from the number supplied, which was constantly nine. Such records furnish an index of the appetite of *Didinium* and have value in determining the relative vitality at different times and under different treatment.

The slight variations in the daily division rate and other measures of vitality, make it difficult to note changes in vitality from time to time, hence it is customary in culture work to make averages of the daily records for periods of 5 or 10 days. This is done by averaging the daily records of all lines of a series for each day, and then averaging these daily averages for the unit periods desired. For the present work, five-day periods have been used.

To test the effects of different tissues on the vitality of *Didinium*, experiments were made lasting for 15 days each, with cancer mush, normal epithelium, and embryonic epithelium, the material in all cases coming from mice under observation in the Crocker Research Laboratory. The tissues were prepared for use in the experiments by Dr. F. D. Bullock, whose valuable assistance I gratefully acknowledge.

Owing to a period of depression and high death rate in the normal cultures and to putrefaction due to heat at the time of the experiments with embryonic tissue, I shall omit these experiments in the present paper.

### *2. The cancer material*

For the cancer work, it was planned to use rapidly growing, non-necrotic tumors of approximately the same age and from the same strain. Mice were inoculated with a high percentage tumor strain (adeno-carcinoma) at such times as to give tumors of approximately the same age, size, and weight for each day of the test. The actual age of the tumors that were used varied from 17 to 21 days and no tumor was used if evidences of necrosis were present. A fresh tumor was used every day of the experimental period of 15 days. After excision, it was trimmed of adipose and external connective tissue, and minced to a fine mush in a chopping machine, all under sterile conditions. This material was then sent directly to my laboratory at the same hour each day. A standard dosage of this mush was obtained by means of a fine platinum wire loop and left for 24 hours in the culture dish with the *Didinium* and *Paramecium*. The cultures were all examined five hours after treatment and records made

of the number of divisions, the number of living *Paramecium* and the number of deaths or of encystment. Similar records were made at the end of 24 hours.

### B. EXPERIMENTS AND RESULTS

*1. Comparison of cancer-treated and control lines of Didinium*

*a. The control series A and B.* In order to get the control material in good running condition before beginning experiments, *Didinium* was cultivated under the laboratory conditions for a period of one month before the first cancer treatment. The variations in the division rate were rather extensive at first, showing that the organisms were not fully adjusted to the conditions of the laboratory cultures. The death rate, also, ran up to 28 per cent at this time, owing chiefly to the number of encystments which occurred during the early stages as a normal reaction to adverse conditions on the part of the organisms. An encysted individual has the same effect on the normal division-rate as a dead one, and for this reason the two are grouped together.

*b. The cancer series C, D, and E.* For the cancer experiments normal individuals were taken from the control series on December 1 and were continued on their normal diet of *Paramecium* plus the cancer mush for a period of 15 days. The cancer tissue was used in three strengths, a single dose being given to a series of five lines derived from control series B; this is described as the C series. A double dose was given to five lines derived from control series A, and described here as series D. A half dose was given to five lines derived likewise from control series A, and described here as series E. In all cases, the experimental animals received a daily dose of the appropriate strength.

The treatment was given in three periods of 5 days each, from Monday to Friday inclusive. On the sixth day of such a period, the ciliates were placed in the normal medium of spring water and given the usual number of *Paramecium*, but without any cancer tissue. This method was adopted to see if there was any after effect of the 5-day treatment with cancer. On the following Monday, the same individuals were again treated with the cancer mush.

Table I gives a summary of the 15 days' treatment. It shows the relations between the division rates of the controls and of the cancer treated animals, during treatment and also in the intervals between treatments.

TABLE I

*Effects of cancer mush on the division rate of Didinium*

| | | FIRST 5 DAYS | SECOND 5 DAYS | THIRD 5 DAYS | TOTAL 15 DAYS | AFTER EFFECTS |
|---|---|---|---|---|---|---|
| Control Series A | Live Didinium | 24 | 19 | 20 | 63 | 8 |
| | Divisions | 63 | 51 | 47 | 161 | 20 |
| | Division rate | 2.62 | 2.68 | 2.35 | 2.55 | 2.50 |
| | Dead Didinium | 1 | 6 | 5 | 12 = 16% | 7 = 46.6% |
| Control Series B | Live Didinium | 23 | 18 | 15 | 56 | 7 |
| | Divisions | 62 | 47 | 30 | 139 | 18 |
| | Division rate | 2.69 | 2.61 | 2.00 | 2.48 | 2.59 |
| | Dead Didinium | 2 | 7 | 10 | 19 = 25.3% | 8 = 53.3% |
| Cancer Series E half dose | Live Didinium | 23 | 17 | 24 | 64 | 15 |
| | Divisions | 67 | 50 | 54 | 171 | 44 |
| | Division rate | 2.91 | 2.94 | 2.25 | 2.67 | 2.93 |
| | Dead Didinium | 2 | 8 | 1 | 11 = 14.6% | 0 |
| Cancer Series C single dose | Live Didinium | 19 | 16 | 21 | 56 | 15 |
| | Divisions | 37 | 29 | 37 | 103 | 49 |
| | Division rate | 1.95 | 1.81 | 1.76 | 1.84 | 3.26 |
| | Dead Didinium | 6 | 9 | 4 | 19 = 25.3% | 0 |
| Cancer Series D double dose | Live Didinium | 9 | 16 | 20 | 45 | 0 |
| | Divisions | 12 | 23 | 27 | 62 | 0 |
| | Division rate | 1.33 | 1.44 | 1.35 | 1.38 | 0 |
| | Dead Didinium | 16 | 9 | 5 | 30 = 40.0% | 15 = 100% |

Series E came from Control Series A; Series C from Control Series B; Series D from Control Series A. "After Effects" are the records, summarized, for two-day periods, following each 5-day test; during these periods the organisms were kept in pure water, and received only one day's food, thus undergoing partial starvation.

Analysis of Table I shows clearly that the cancer mush had a marked effect on *Didinium*. With the single dose, the division rate was 1.84 per day per individual for the entire 15 days. This series (C) was taken from the control series B in which the division rate was 2.48 for the same period. The death rate for

both series was the same, 25.3 per cent. The result indicates a well-marked depressant effect on vitality.

This depressant effect is still more strongly indicated in series D in which the individuals were treated with a double dose. The division rate here was 1.38 and the death rate 40 per cent, while the control series A from which the double dose series was taken had a division rate of 2.55 and a death rate of 16 per cent.

With the half dose, however—series E—the results, while strongly marked, were quite different in nature. Instead of a depressant effect there was a well-marked stimulation as shown by the division rate of 2.67 and a death rate of 14.6 per cent—the control series A from which this set was taken having a division rate of 2.55 and a death rate of 16 per cent.

In computing these tables I have considered the division rate and the death rate separately, whereas both should be taken together for a true index of vitality. This is the usual method employed in culture work and is usually represented graphically by a curve in which the ordinates represent the average division rate obtained by dividing the total number of divisions by the total number of individuals isolated, for the unit period, while the abscissae represent the successive unit periods. The accompanying diagrams show the effects of treatment in daily averages for the entire period.

The division rate of the half dose series E averaged the same as, or higher than, the control A on 9 days, and lower than the control on 6 days out of the 15, giving a general average for the 15 days of 2.28 per day as against 2.14 for the control.

The division rate of the double dose series D was the same as, or higher than, that of the control series A on 3 days only out of the 15, and was lower on 12 days with a general average for the 15 days of 0.82 as against 2.14 for the control.

The results from this set of experiments indicate two conclusions—first, that the cancer tissue used contains something which produces a depressant effect on *Didinium*, and second, that the cancer tissue used contains something which produces a stimulating effect on *Didinium*. I shall speak of the former as the *lethal factor*, and of the latter as the *stimulating factor*.

Table I shows that the double dose series D had a death rate of 40 per cent. Preliminary experiments with doses larger than the double dose were invariably fatal, both for *Didinium* and for *Paramecium*, hence the dosage used in series D may be regarded as sub-lethal with the depressant effect shown by the high death rate and the low division rate.

The stimulating factor is less evident, and the general results taken by themselves might be well within the limits of experi-

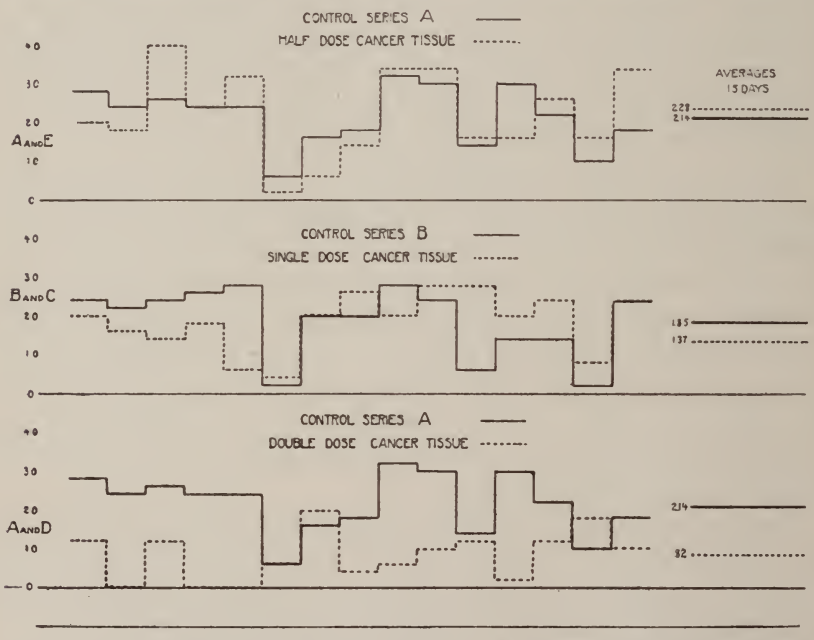


Diagram 1. Average division rates (daily) of five lines each of control series (solid lines) and series treated with cancer (broken lines). On the right are the average division rates for the entire fifteen days.

mental error. It must not be overlooked, however, that any excess in the division rate of cancer-treated individuals occurs in spite of the adverse action of the lethal factor, for they have directly opposite effects on the vitality of *Didinium*. In weak doses the stimulating factor is apparently more effective than the lethal factor and there is some very slight evidence to show that *Didinium* acquires some immunity to the latter. Thus in

the single dose series C the division rate was the same as, or higher than, that of the control series B every day of the last six days, and lower every day for the first five days, while in the intermediate days it was sometimes higher, sometimes lower, the averages for the last 10 days being 2.02 for series C and 1.54 for control series B. Yet the average for the 15 days was lower than the control. (Diagram 1.)

Additional evidence of the stimulating factor in cancer tissue is shown by (1) the after effects of treatment, and (2) effects during the first five hours after daily treatment. The after effects are indicated by the division rate during the two days in spring water after five days' continuous treatment with cancer. Three such periods are averaged in the last column of Table II. The division rates of the 15 individuals of each series averaged for the 6 days were control A—2.50, control B—2.59, single dose series C—3.26, half dose series E—2.93, and double dose series D—0. The high death rate of the controls, 46.6 per cent and 53.3 per cent, was due to the fact that all of the cultures were left for 48 hours without fresh water and food instead of 24 hours as usual. This very fact, however, supports the evidence in favor of a stimulating factor, for the same treatment was given all series, C and E going 48 hours without change, as well as A and B. The death rates, however, were nil, not a single cancer-treated individual of the C and E series dying during these after-treatment periods. (Table I.)

The after effects of treatment with a double dose of cancer, however, were quite different. Not a single one of the 15 lived through the trying period of 48 hours after treatment, the series being continued each time from individuals of the C series.

Except for the double dose series, therefore, where the lethal factor had weakened vitality to such an extent that all individuals succumbed to the adverse conditions which carried off more than half of the control animals, there is strong evidence here to support the conclusion that a stimulating factor is present in cancer tissue.

Further support of this conclusion is given by the results obtained from records made 5 hours after treatment and summar-

ized in Table II. As the 5-hour records were not made for the first 3 days the summary is based on the last 12 days of the 15-days of treatment.

There is little room for doubt of the stimulating effect of cancer tissue during the first 5 hours after treatment. It is shown both by the division rate and by the appetite. Thus 16 of the 60 isolated in the control series A divided, while 32 out of 60 in the half dose cancer series divided, and 27 and 26 in the double dose and single dose series respectively. Control series B ate

TABLE II

*Effects of cancer tissue on vitality of Didinium; first 5 hours*

| | | | SERIES A | SERIES B | SERIES C | SERIES D | SERIES E |
|---|---|---|---|---|---|---|---|
| | | | Control | Control | Single dose | Double dose | Half dose |
| Summary of records made 5 hours after treatment. 12 days | Didinium | Number isolated.... | 60 | 60 | 60 | 60 | 60 |
| | | Number of divisions in 5 hours... | 16 | 18 | 26 | 27 | 32 |
| | | Percentage of divisions..... | 26.6% | 30.0% | 43.3% | 45.0% | 53.3% |
| | | Percentage of deaths..... | 5.0% | 3.3% | 6.6% | 0 | 8.3% |
| | Paramecium | Number supplied.... | 540 | 540 | 540 | 540 | 540 |
| | | Number alive at end of 5 hours.... | 406 | 449 | 388 | 381 | 406 |
| | | Percentage eaten.... | 24.8% | 17.0% | 28.1% | 29.4% | 24.8% |
| | | Percentage of deaths..... | 0 | 0 | 0 | 0 | 0 |

17 per cent of the *Paramecium* supplied them, while series C derived from B ate 28.1 per cent of the *Paramecium* supplied in the same time. It is significant that in the double dose series D there is well marked evidence of the stimulating factor as shown by 29.4 per cent, indicating that more *Paramecium* were eaten than in any other series. The lethal factor is not in evidence during the first few hours of treatment as shown by these high percentages and by the low death-rate (0). That it is not absent, however, but only masked by a possible excess of the stimulating factor due to the large quantity of tissue used, is shown

by the high death rate and low division rate in this series after 24 hours treatment.

Taking all the evidence into consideration, there is a good basis for the conclusion that Woodruff and Underhill were right in finding that cancer tissue may produce both depressant and stimulating effects on living indicators. The former may be due to what I here term a lethal factor, the latter to a stimulating factor. Evidence of the lethal factor is seen in the fact that large doses kill, while weaker doses cause depressed vitality and an abnormal death rate, and fatal after-effects. Evidence of the stimulating factor is seen in the increased division rate and decreased death rate with minimum doses; in increased vitality with all doses up to five hours after treatment, indicated by increase of appetite and increase of division rate; and in well marked after-effects on division and death rates, indicating stimulation.

### *2. Comparison of control series with series treated with normal epithelium*

While there is no doubt that cancer tissue produces well marked effects on living indicators, it remains to be shown whether such effects are different in kind or in degree from effects produced by normal epithelium used in the same way. Taken alone, the results of the cancer experiments might be interpreted as effects produced by the same factor which, like many poisons, stimulates when used in weak doses, depresses or poisons when used in stronger doses. It is important to note, therefore, whether normal epithelium has a lethal effect alone, a lethal plus a stimulating effect, a stimulating effect alone, or has no effect at all on the vitality of *Didinium*. It would have been better to carry on this control experiment at the same time with the cancer experiments, but the techical difficulties were so great that only one set could be carried on at a time.

A period of two weeks elapsed from the end of the cancer experiments to the beginning of the normal epithelium experiments. During this period the race of *Didinium* underwent encystment with complete cellular reorganization and were in a period of

high vitality marked by high division rate and low death rate at the time when the normal tissue experiments were begun (Calkins, loc. cit. p. 233).

The normal tissue used was obtained by first shaving the abdomen of a mouse, excising a piece of the skin, and grinding it in a mincing machine, all under sterile conditions. This was brought immediately to my laboratory and used at once. Fresh material prepared in this way was used daily for a period of fifteen days. Three dosages of the same strength as those of the cancer experiments were used in three series of five *Didinium* each, all derived from the control series A and B. A single dose was given individuals of series C, a double dose to series D, and a half dose to series E. Each individual isolated received, as before, nine *Paramecium caudatum*. The results, summarized and tabulated as for the cancer experiments, are shown in Table III.

In analyzing this table it must be remembered that the race was in a stage of high vitality with a division rate almost as high as it can go, for growth and division have their normal limits. A slight increase, therefore, in the rate of division has much significance.

The controls averaged—A, 2.67 and B, 2.79 divisions per day for the 15-day period. The single dose series C averaged 2.79 divisions per day while the control series A from which the C series was taken averaged 2.67. The double dose series, also from control A, averaged 2.64 and the half dose series E averaged 2.95 while the control series B from which E was taken averaged 2.79. There was therefore a distinct increase in the division rate of all the individuals treated with normal tissue except the double dose where in the first 5-day period it fell so far below the control (2.48 and 2.68) that the average for the 15 days fell below the control. In the other two 5-day periods, however, the division rate of series D was above the control (2.64 against 2.52 and 2.80 against 2.63), so even with the double dose the stimulating effect is evident.

The stimulating effect of the normal tissue is shown also by the decrease in the death rate. Owing to the high vitality of

the race at this period the death rate of the controls was low, 10.6 per cent for both A and B controls, but with the tissue-treated individuals it fell to 6.6 per cent for the double dose series D, to 4 per cent for the single dose series C, and to 2.6 per cent for the half dose series E.

TABLE III

*Effects of normal epithelium mush on the division rate of Didinium*

| | | FIRST 5 DAYS | SECOND 5 DAYS | THIRD 5 DAYS | TOTAL 15 DAYS | AFTER EFFECTS |
|---|---|---|---|---|---|---|
| Control Series A | Live Didinium | 22 | 23 | 22 | 67 | 11 |
| | Divisions | 63 | 58 | 58 | 179 | 31 |
| | Division rate | 2.86 | 2.52 | 2.63 | 2.67 | 2.82 |
| | Dead Didinium | 3 | 2 | 3 | 8 = 10.6% | 4 = 26.6% |
| Control Series B | Live Didinium | 24 | 23 | 20 | 67 | 11 |
| | Divisions | 62 | 64 | 61 | 187 | 36 |
| | Division rate | 2.59 | 2.78 | 3.05 | 2.79 | 3.27 |
| | Dead Didinium | 1 | 2 | 5 | 8 = 10.6% | 4 = 26.6% |
| Tissue Series E half dose | Live Didinium | 23 | 25 | 25 | 73 | 14 |
| | Divisions | 65 | 73 | 78 | 216 | 42 |
| | Division rate | 2.82 | 2.92 | 3.12 | 2.95 | 3.00 |
| | Dead Didinium | 2 | 0 | 0 | 2 = 2.6% | 1 = 6.6% |
| Tissue Series C single dose | Live Didinium | 24 | 24 | 24 | 72 | 15 |
| | Divisions | 70 | 69 | 62 | 201 | 49 |
| | Division rate | 2.91 | 2.87 | 2.50 | 2.79 | 3.27 |
| | Dead Didinium | 1 | 1 | 1 | 3 = 4% | 0 |
| Tissue Series D double dose | Live Didinium | 21 | 25 | 24 | 70 | 15 |
| | Divisions | 52 | 66 | 67 | 185 | 52 |
| | Division rate | 2.48 | 2.64 | 2.80 | 2.64 | 3.55 |
| | Dead Didinium | 4 | 0 | 1 | 5 = 6.6% | 0 |

Series E came from Control Series B; Series C and Series D from Control Series A. "After Effects" are the records summarized for two-day periods after each 5-day test; during these periods the organisms were kept in pure water and received only one day's food thus undergoing partial starvation.

The curves of vitality for all lines averaged daily and including both divisions and deaths are shown in Diagram 2.

The stimulation is also shown in the after effects when the individuals were subjected to starvation for 24 hours. As with the cancer experiments, after each period of 5 days the controls

and the experimental animals were placed in pure water and were each supplied with 9 *Paramecium caudatum*, or one day's ration. They were then left for 48 hours and were thus partially starved the last day. The results are given in the last column of table III where the division rate and the death rate for the six days are averaged for 48 hours. The controls divided, on the average, 2.82 and 3.27 times in the 48 hours, while the

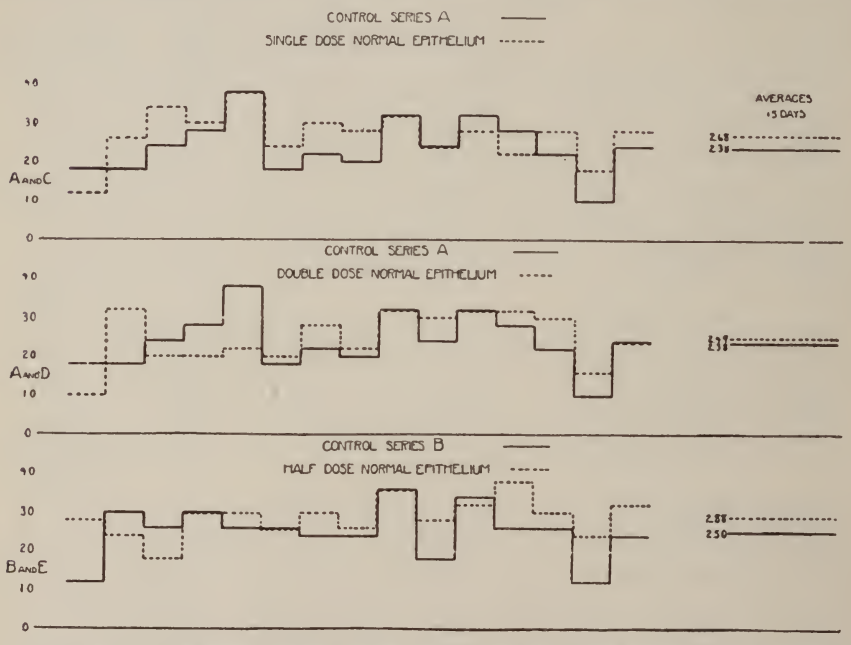


Diagram 2. Average division rates (daily) of five lines each of the control series (solid lines) and series treated with normal epithelium (broken lines). On the right are the average division rates for the entire fifteen days.

death rate was 26.6 per cent in each case. The tissue-treated individuals of series C, D, and E divided at the same periods 3.27, 3.55, and 3.00 times, with death rates of 0 per cent, 0 per cent and 6.6 per cent. The tissue-treated individuals therefore were more vigorous and better able to withstand the adverse conditions than were the controls.

The effects of the stimulation are also shown by the appetite up to 5 hours after treatment, as shown in Table IV.

This table explains itself: not only was there an increased percentage of divisions in the majority of tissue-treated individuals, but the percentage of *Paramecium* eaten, or the appetite, was greater in all tissue-treated individuals than in the controls.

These various records leave little reason to doubt the fact that normal epithelium of the mouse, like cancer tissue of the mouse, contains a stimulating factor. Unlike the cancer tissue, however, there is absolutely no evidence of the presence of a lethal factor in the normal tissue. The death rates, even with the stronger

TABLE IV

*Effects of normal epithelium on vitality of Didinium; first 5 hours*

Summary of records made 5 hours after treatment. 15 days

| | | SERIES A | SERIES B | SERIES C | SERIES D | SERIES E |
|---|---|---|---|---|---|---|
| | | Control | Control | Single dose | Double dose | Half dose |
| Didinium | Number isolated.... | 75 | 75 | 75 | 75 | 75 |
| | Number of divisions in 5 hours... | 31 | 37 | 45 | 41 | 27 |
| | Percentage of divisions..... | 41.3% | 49.3% | 60.0% | 54.6% | 36.0% |
| | Percentage of deaths..... | 0 | 0 | 0 | 0 | 0 |
| Paramecium | Number supplied.... | 675 | 675 | 675 | 675 | 675 |
| | Number alive at end of 5 hours.... | 555 | 544 | 490 | 506 | 511 |
| | Percentage eaten.... | 17.8% | 19.4% | 27.4% | 25.0% | 24.3% |
| | Percentage of deaths..... | 0 | 0 | 0 | 0 | 0 |

doses used, were invariably less than the controls. These facts show also that the stimulating and the lethal factors are not due to the same thing in different strengths. Thus the half dose series both with cancer and with normal tissue gave the greatest increase in the division rates over the controls. The double dose with cancer gave a mortality of 40 per cent or more than twice that of the control (16 per cent), while the double dose with normal epithelium gave a mortality of 6.6 per cent against 10.6 per cent for the control. With four times as much tissue material in the double dose series as in the half dose, the death

rate with normal epithelium was not raised even to that of the normal controls—hence the lethal factor cannot be due to an excess of the stimulating factor.

### C. CONCLUSIONS

One conclusion to be drawn from these experiments stands out prominently, viz., all epithelial tissues used in the manner described have a stimulating effect on the normal free-living indicator organism, increasing its vitality and causing it to reproduce more rapidly than the control organisms. A second conclusion, equally definite, may be drawn from a comparison of the results with cancer tissue and with normal epithelium, viz., the presence of a lethal factor in cancer tissue and its absence in normal tissue.

First let us examine this so-called lethal factor in cancer tissue. Is it the same as, or different from, some putrefactive factor? This is a hard question to answer and involves chemical analyses of such delicacy and difficulty that no attempts were made to carry them out. External signs of putrefaction such as indicated bacterial decomposition in similar tissue in experiments made during warm weather in May, were absent in the cancer tissue cultures during the cold weather of December. Furthermore, the living indicators were killed in the fresh cancer mush given in large doses. Again, if putrefaction, although not indicated by gross appearance and odor, were the cause of the high mortality with cancer tissue, how can we account for the absence of putrefaction and low death rate in the experiments with normal epithelium, also carried out in cold weather (January)? So far as the conditions of the cultures were concerned, there was as much reason for putrefaction of the teased normal epithelium as of the teased cancer tissue, and yet the death rates of all the series treated with such normal tissue were lower than the death rates of the control series. Cancer tissue disintegrates and breaks down chemically more quickly than does normal tissue and also contains more or less necrotic material, no matter how firm and solid the tumor appears on excision, and there is probably more rapid bacterial action than in normal tissue, but I do

not think that putrefaction was the cause of the high mortality of the cancer series. The lethal factor, I believe, is a product of necrosis or of the chemical break-down of protoplasm, and is not present ordinarily in normal adult tissue.

The stimulating factor, found in all tissues, was an unexpected result of the experiments. We were led to infer from Woodruff and Underhill's results that a stimulating effect was produced by cancer tissue alone, and not by normal epithelium; but their paper is a little obscure with respect to this point, since no comparisons were made with the normal indicators on their natural diet alone. However, the results of our experiments leave no room to doubt the fact that normal tissue as well as cancer tissue contains something that stimulates the vital activities of the normal indicator, and it is probable that this "something" is the same in all cases. Is there any evidence as to its nature? There is considerable license involved in using the expression "normal" tissue in connection with these experiments—very little of the "normal" remains when a tissue is chopped fine and placed in water. Autolytic processes begin at once and a chain of chemical compounds of more or less unstable nature are formed, from nucleins and nucleo-proteins down to the late products of protoplasmic disintegration. Just what these are I do not know, but they may be of the same nature as those formed during the multiple processes of destructive metabolism. Some of these are crystalloids which are kept for a longer or shorter time in solution in water. Amongst such formed products of protoplasmic breakdown, there are some which are known to be stimulants to the growth and division of cells.

For example, the cells of a plant are stimulated to division by a nucleo-protein poison injected by an insect, and a gall results. Again, some of the purine derivatives, coming from the chemical breakdown of nucleins act directly as stimulants to cell division. This was shown by experiments with *Actinobolus* carried out three years ago in which some twenty fixed products of protoplasm breakdown were used. While some of these had no effect, and others a lethal effect, some—xanthine and hypoxanthine in particular—had a well marked stimulating effect (Calkins, Bullock, and Rohdenburg, *Jour. Infect. Dis.*, 1912, x, 421).

There is some indirect evidence that similar products of protoplasmic activity play an important part in normal cell-division. The infusorian *Uronychia transfuga* has relatively enormous motile organs in the form of posterior cirri. Individuals were cut transversely with a scalpel at different periods after division and in such a way that the micronucleus was present in the posterior portion and, of course, absent in the anterior part. The posterior part always regenerated but the anterior part without a micronucleus never regenerated if the cells were cut from 15 minutes to 18 hours after division. Uronychia divides about once in 26 hours, and if it is cut during the latter part of the inter-divisional period from 18 to 24 hours after division or just prior to the next division, both parts regenerate perfectly except that the anterior part contains no micronucleus. The same result is obtained if the cells are cut in such a way that the micronucleus is in the anterior part, or on the right side, or on the left side; the enucleate fragment never regenerates unless the cell is cut just prior to division, when regeneration is perfect (Calkins, *Jour. Exp. Zool.*, 1911, x). These experiments indicate the accumulation in the cell of something which determines regeneration. Just prior to the normal division process with its accompanying regeneration processes this "something" is distributed all through the cell, inasmuch as any part will regenerate, but it is exhausted or used up with the processes of normal division, for no regeneration occurs if the cell is cut immediately after division. We have no evidence as to what this substance is, but the phenomenon is an observed fact which may be interpreted hypothetically as due to the accumulation of products of destructive metabolism. If such products are introduced into the cell from external sources we should expect a more active division rate. This was done with *Actinobolus* and, as stated above it was found that xanthine and hypoxanthine have such an effect.

It is a far cry from protozoa to cancer, but the cancer problem is fundamentally a cellular problem; and it is justifiable to compare the biological activities of cells, whether they are protozoa or tissue cells. Is there anything in pre-cancerous conditions to indicate an accumulation of products of destructive metabolism

or of autolysis which will act as externally introduced stimulants on the normal cells?

We have such a clue to the causation of cancer in the well known facts linking up chronic irritation with pre-cancerous and cancerous conditions. The Kangri-burn cancer in natives of Kashmir is an epithelioma of the abdomen due to more or less chronic burning from the charcoal fire carried in the Kangri basket for warmth; the X-ray cancer is due to superficial irritation by burning. Similarly, cancers are traced to betel nut chewers, hot rice eaters, clay pipe smokers, etc.—all following chronic irritation. Or cancers may be traced back to chronic irritation due to external or internal parasites as in the case of the primary tumors of rodents observed in numerous laboratories. Thus Borrel found a path of inflammatory tissue running from an acarid parasite of a mouse back to the original site of the parasite where an active primary tumor was growing. It is conceivable that other types of cancer, like those of the mammary glands, the uterus, intestine, etc., are due to chronic irritation of one kind or another and for which there is more or less evidence.

Chronic irritation means killing of cells more or less continuously in a localized area. Localized centers of autolysis, or protoplasmic breakdown, are formed and the products are absorbed by the surrounding normal cells and by the blood. Some of these products, as we have seen, have a stimulating effect on normal cells; these divide and the process of regeneration is started. This is indicated in the *Didinium* experiments with normal epithelium up to 5 hours after treatment (Table V) during which time autolysis was slow. The larger the dose, however, the more effective were the products during this period. Thus the division rates were 36 per cent for the half dose series, 54.6 per cent for the double dose, and 60 per cent for the single dose, while the controls were 41.3 per cent and 49.3 per cent. It is also well illustrated by experiments of Bullock and Rohdenburg in my laboratory, in which the posterior lobes of the pancreas in rats were tightly ligatured so that the blood and food supply were cut off. These were then left to autolyze in situ. The rats were killed at intervals of 5, 10, 15, and 20 days and the

adjacent regions of the normal pancreas were sectioned. Hundreds of mitotic figures were found in zones in the vicinity of the ligatured portion, indicating what is generally interpreted as a normal regeneration process. But what is regeneration and what calls it forth? I believe it is due to the stimulus of chemical products of autolyzing dead protoplasm.

With chronic irritation we have the continued repetition of this process of cell death and regeneration from surrounding cells, until hyper-regeneration results and a mass of cells of like kind is formed, and added to by continuation of the process. Central cells of the mass, or others cut off from blood and food supply, autolyze in situ forming centers of necrosis, and the tumor, having reached this stage, is self-perpetuating. The products of necrosis, in addition to the stimulating effect on normal vitality, also include lethal factors capable of producing the morbid symptoms in the organism as a whole, and capable also of cytolytic functions whereby cell boundaries are dissolved, thus providing the invasive power so characteristic of malignant tumors. Unless the mass of cells is enclosed by a protecting connective tissue capsule, a cancer is the result.

I have attempted here to give only in the briefest outline a theory of the cause of cancer. It has as a slender basis the effects of products of autolyzing tissue cells on the vitality of living indicators, plus the well-known connection between chronic irritation and certain types of malignant tumors. To prove or disprove each step empirically will be the work of years, but an advance step in this direction is now under way in the attempt to localize experimentally the factors in autolyzing tissue capable of stimulating cellular activities, and to distinguish them from the lethal factors.

# THE EFFECT OF PHLORHIZIN ON TUMORS IN ANIMALS

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In the first number of this Journal, Wood and McLean (1) have published a paper detailing experiments to study the action of phlorhizin upon the growth of experimental and spontaneous tumors in rats and mice. This work, which was carried out upon a large scale, was instituted to test the correctness of the work and conclusions reported by Benedict and Lewis (2) under the title "The Influence of Induced Diabetes on Malignant Tumors." As a result of their experiments Wood and McLean conclude that the "cures" obtained by Benedict and Lewis in work with the Buffalo rat sarcoma must be ascribed to spontaneous absorption, and not to the therapeutic agent.

It is the opinion of the present writer that the experiments reported by Wood and McLean have no direct relationship to the work of Benedict and Lewis. These latter authors studied the effects of a complete diabetes, induced by large doses of phlorhizin, upon the growth of experimental malignant tumors. Their experiments were designed to test the possibility of growth of tumors when the cells were unable to utilize any glucose whatever. Wood and McLean, on the contrary, simply studied whether traces of phlorhizin introduced into animals would influence the growth of tumors. The negative results they obtained were to be expected, since there is nothing in the work of Benedict and Lewis, or elsewhere, to lead to the belief that phlorhizin, in the doses employed by Wood and McLean, would affect the growth of tumors or other tissues.

The dose of phlorhizin employed by Wood and McLean[1] (3 mgm. for each rat once in two or three days) was less than one-sixtieth of that employed by Benedict and Lewis. Wood and McLean state that they tried larger doses, but that their animals died so rapidly that they had to reduce the dose to the amount indicated.

It is plain that since Wood and McLean used a dose of phlorhizin which was less than one-sixtieth of that employed by Benedict and Lewis, their work does not bear directly upon that of these latter investigators. The fact that Wood and McLean were able to detect sugar in the urines of their animals receiving traces of phlorhizin does not indicate that such animals were not utilizing large amounts of glucose. Minimal doses of phlorhizin, down to an almost infinitely small amount will produce glycosuria, but for a complete diabetes a definite maximal dose is necessary. These relations have been discussed in a recent résumé by Lusk (3). Although not reported in their paper, Benedict and Lewis tried smaller doses of phlorhizin than the 200 mgm. regularly used in their work. In spite of the fact that these doses were some thirty times as large as those employed by Wood and McLean, they were without any appreciable effect upon the tumor growth.

In their work with phlorhizinized rats Benedict and Lewis made use of adult, apparently strong animals, with large and rapidly growing tumors prior to beginning treatment.[2] Even with such animals there is a considerable mortality, and in almost no cases can the treatment be continued much over ten days

[1] On page 52 in their article Wood and McLean state that for rats they employed 3 mgm. of phlorhizin for each animal, but in the experiment detailed on page 60 they apparently used only 1 mgm. for each animal.

[2] A few trials were made by Benedict and Lewis in which phlorhizin was given simultaneously with the tumor planting. The results seemed to indicate that under such conditions the tumor cells may adjust themselves to the altered conditions of nutrition much better than can those of a large and rapidly growing tumor, so that phlorhizin may be far less effective in the former cases. As a rule the larger and more rapidly growing a tumor is when phlorhizin administration is begun, the more rapid and marked is the effect of the drug. Such a result is quite in harmony with the view that phlorhizin acts upon tumors in rats only through influencing their nutrition.

without intervals of a few days when the animal is fed upon bread and the administration of phlorhizin is discontinued. The animals employed by Wood and McLean were evidently unsuited to the strenuous treatment with phlorhizin and a carbohydrate-free diet, as is witnessed by the high mortality which occurred even without any treatment.[3]

In conclusion it is desired to correct some misstatements concerning the work of Benedict and Lewis which appear in the paper of Wood and McLean. These latter authors state that as a result of the work of Benedict and Lewis "a case of sarcoma in man was treated with phlorhizin with an unfavorable result." In the case reported by Benedict and Lewis no mention was made of an "unfavorable" result. The patient treated said that he suffered less pain during the treatment, and lived for some months after it was discontinued. He ultimately died from pneumonia—a usual termination in a case with an infected growth in the mouth.

Wood and McLean further state that Benedict and Lewis "gave no charts to show whether or not those tumors which disappeared under treatment had already begun to decrease in size when the injections of phlorhizin were started." It is true that Benedict and Lewis gave no charts such as Wood and McLean have offered, but they did cite a specific example of a growth in which the measurements showed continuous and rapid growth up to the time of beginning treatment, and a continuous decrease in size after treatment was inaugurated. It was also stated that this example was one of several similar ones.

Wood and McLean state that "the largest tumor reported as cured by Benedict and Lewis . . . . measured 20 x 25 mm.," and that they themselves noted spontaneous retrogression in a growth almost as large. The following is a quotation from the paper of Benedict and Lewis. "The largest growth which we

[3] Wood and McLean employed meat and lard as a diet for their experimental animals. Such a diet is probably less suited to rats than is the one of casein and lard, which Benedict and Lewis used. (In the oral presentation of their paper Benedict and Lewis mentioned specifically the diet which they employed, but this was inadvertently omitted from their published paper.)

have succeeded in curing by the above indicated treatment measured 45 x 47 mm. We believe that this is by far the largest experimental growth which has so far been successfully treated."

BIBLIOGRAPHY

(1) Wood and McLean: Jour. Cancer Research, 1916, i, 49.
(2) Benedict and Lewis: Proc. Soc. Exp. Biol. and Med., 1914, xi, 134.
(3) Lusk: Ergebnisse der Physiologie, 1912, xiii, 315.

## IN REPLY TO DR. BENEDICT

F. C. WOOD

The Editors of the Journal of Cancer Research have, with the approval of the author, courteously allowed me to read the proof of Prof. Benedict's rejoinder to a paper by Dr. E. H. McLean and myself, in order that any reply might be printed with it.

I find nothing in Prof. Benedict's paper which, in my opinion, invalidates the conclusion published by Dr. McLean and myself, that phloridzin in sublethal amounts has no therapeutic value in the treatment of either carcinoma or sarcoma in animals.

# STUDIES ON THE CROWN GALL OF PLANTS

## ITS RELATION TO HUMAN CANCER

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The present paper reviews certain of the essential features which characterize the growth of crown gall, especially as they bear upon the general problems of cancer. In addition, it contains a number of new observations, which appear to bring this vegetable growth into relation with the group of tumors described as embryomata.

### II. CROWN GALL

In brief, crown gall as here described is a plant disease, tumor-like in nature, which is produced by a bacterial organism. It is a growth very common in many parts of the world (North America, South America, Europe, Asia, Africa) on a great variety of cultivated plants, and on some wild ones. It is known by many names, but generally in the United States as "crown gall," because it occurs very often on what the gardeners call the crown of the plant, i.e., on that part where stem and root join, but it may occur on any part of the plant (Pl. I, figs. 1 and 2).

### III. NATURE OF THE TUMOR

Arguments against the cancerous nature of plant tumors based upon conditions in those tumors of turnips and cabbage which have been most studied by animal pathologists, or in over-growths due to gall-insects, or to various fungi, or even to other bacteria, do not apply to this tumor. Crown gall, is not an hypertrophy due to the enormous enlargement of a few special parasitized cells, like the bacterial root nodules of

legumes or the finger-and-toes of cruciferous plants, ascribed to the slime-mold *Plasmodiophora brassicae*, nor is it a granulomatous hyperplasia in which the bacteria are located in cavities or pockets between the cells and pass by way of the vascular system into distant regions, like the olive tubercle, due to *Bacterium savastanoi*. On the contrary it is a peculiar hyperplasia, the bacteria which are the cause of it developing sparingly and only within the cells, which they compel to divide early and repeatedly, so that a great mass of non-capsulated, small-celled tumor tissue arises (Pl. I, figs. 3 and 4, Pl. II, figs. 5 and 6, and Pl. IV, figs. 17*a*, 18) in which the bacteria themselves are invisible. These numerous, small, incompletely developed, atypical, unripe cells are endowed with enormous reproductive capacity which is not under the physiological control of the plant (Pl. III, figs. 7 to 13 and Pl. IV, figs. 14 to 16—sunflower), and the continued development of which is detrimental to the plant both locally and constitutionally. Various grades of anaplasia exist, some of the cells in the tumor reaching nearly the normal size and form before division, while others divide quickly again and again, remaining quite embryonic. The tumors, even when deep-seated, are incompletely vascularized (Pl. IV, figs. 17, 18, and Pl. VIII, fig. 26), often quite fleshy, and very subject to decay (Pl. V, fig. 20). These conditions naturally result in the production of open wounds, which are subject to a variety of secondary infections, some of them very harmful to the plant. The tumor when inoculated on fleshy roots such as the sugar beet is often larger than the root itself.

Physiologically and structurally the tumor resembles cancer in many ways. As ordinarily seen it certainly exhibits growth independent of function. Vegetative activity is stimulated, functional activity is depressed. The tumor is incompletely vascularized and early central necrosis is common.

It can be stimulated by feeding or starved into quiescence similarly to mouse cancer. It can be grafted on to other plants of the same species. Like cancer, it can be cut out, but it will return if all has not been removed. It often grows with aston-

ishing rapidity and destroys tissues both by compression and by infiltration; there is then surface growth and no capsule. Sometimes the tumor recedes of its own accord, but whatever immunity is worked up in the plant appears to be temporary. The diseased plant may also show something corresponding to cachexia in the animal (Pl. VII, figs. 23 and 24), although generally it is less pronounced than in the animal body. The cells of the tumor are often markedly embryonic in their character (Pl. I, figs. 3, 4, Pl. II, Pl. IV, figs. 17a, 18 and Pl. V, fig. 19), and show in different tumors, and also in different parts of the same tumor, various grades of undifferentiation (Pl. VIII, figs. 25, 26, and 27), the more embryonic cells exhibiting a very strong affinity for stains (Pl. IV, figs. 17*a* and 18, Pl. VIII, fig. 28, and Pl. XXIII). As in cancer (see Adami: Principles of Pathology, vol. I, p. 836), normal cells can be seen actually changing into atypical blastomous cells (margin of figs. 3, 4 and 78).

As in cancer the nuclei of this tumor divide both mitotically and amitotically. Nuclear division by simple cleavage is rather common (Pl. VIII, fig. 29*a*). Variously lobed, notched, and cleft nuclei are as common in crown gall as in animal neoplasms (Pl. VIII, fig. 29*b*). Cells containing several nuclei (2 to 4) are produced in this way and are common (Pl. VIII, fig. 30). More rarely, in tobacco, I have seen 5 to 7 nuclear fragments in a cell, but I have not seen any large multinucleate giant-cells (10 to 30 nucleate) such as always occur in plant tumors due to nematodes, even in the youngest stages. Lignin (wood) may be deposited abnormally on the walls of large parenchyma cells, just as bone is deposited out of place in certain animal cancers. There is a marked disturbance of polarity in cells and vessels. Finally, as there may be an inflammatory reaction around a cancer, so in the vicinity of a crown gall there may be excessive nutrition and growth leading to enlargement of parts not actually parasitized (Pl. III, fig. 12, overgrowth of wood; and Pl. IV, fig. 17, both of wood and of bark; see also Pl. X, fig. 36, where the wood only is involved).

Other histological resemblances are now to be considered.

## IV. OCCURRENCE OF SECONDARY TUMORS AND OF A TUMOR-STRAND

Deep-seated secondary tumors occur frequently (Pl. IX, figs. 31 to 34) often at a considerable distance from the primary tumor and that, too, within a period of time measured only by a few weeks or a few months from the onset. They contain the same intracellular organism as the primary tumors. So far as can be determined these secondary tumors are not produced by the mechanism which obtains in the case of the olive tubercle, caused by *Bacterium savastanoi*, or as human and animal tuberculosis. In both of these diseases the secondary growths are caused by direct bacterial migration either internally through special vascular channels, or externally from ruptured diseased surfaces to fissured healthy surfaces, the bacteria in the new location setting up local irritations that result in inflammatory (granulomatous) cell reactions repeating the old picture of the disease. On the contrary, in crown gall the secondary tumors are outgrowths from a tumor-strand, inasmuch as microscopic examination has shown that they are connected with the primary tumor by means of a chain of tumor cells. This chain, strand, root, or pedicel of bacterially infected cells in which the secondary tumors have their origin is sometimes large enough to be clearly visible to the naked eye on cross-sections of stems made between the primary and the secondary tumor (Pl. X, fig. 36), but more often it consists of a delicate thread of cells and vessels (Pl. X, figs. 35, 37 and 38) sometimes hard to find even under the microscope and then best seen in longitudinal sections (Pl. X, figs. 39, 40, and 41, and Pl. XI, fig. 42) or else in a long series of cross-sections, owing to the fact that even when the strand is destitute of trachei and is reduced to a minimum number of cells in a particular region it is usually wider and clearly visible just above or below this region, just as a tumor-strand in animals may be obliterated in places by fibrous ingrowths, to appear again farther on. In tobacco stems I have seen this tumor-strand only in the outer bark (cortex); in stems of the Paris daisy it crowds its way

through the region of the pith periphery, usually between spiral vessels (Pl. X). Wherever it occurs it is clearly differentiated from the tissues surrounding it both in structure and in reaction to stains. I have traced it for a distance of 8 inches from the primary tumor in the Paris daisy and for a like distance in the sunflower, but I regard the stem of the Paris daisy as better adapted to the study of strand formation than the disk of the sunflower.

Some additional examples of infiltration are shown on Plate XIII. Figure 51 of this plate shows the margin of a secondary tumor in the petiole of a Paris daisy where small-celled, atypical, blastomous tumor tissue, not well vascularized, is growing toward the surface of the plant between large cells of the petiole parenchyma. The normal tissue is under pressure and curved outward. Figure 52 shows the margin of a primary tumor in the sunflower. Here the small-celled blastomous tissue, which in this case is remarkably well vascularized, can be seen pushing out between the large cells of the pith. The color contrast shows the varying reaction to stains. Some of the vessels (trachei), as at *X*, are very thin-walled and still too young to take the lignin stain. A third example of an invasive strand, taken from the hothouse geranium (Pelargonium), is shown on Plate XVI. I have also seen and figured a similar strand of fine-celled blastomous tissue extending from a small tumor into the coarse-celled cortex of a young tobacco stem (Bull. 255, Pls. 102, 103). See also Plate XX.

A true metastasis does not occur in plants, because their structure (of rigid cell-walls) does not lend itself, like that of animals, to this form of tumor propagation.

## V. STRUCTURE OF THE SECONDARY TUMORS

One of the striking features of cancer, separating it sharply from all other animal diseases, is the fact that the secondary tumors are not granulomatous proliferations.

In this particular, again, these plant tumors resemble cancer in man and the lower animals, since the secondary tumors are

not granulomatous proliferations but reproduce the structure of the primary tumor. Thus, when a primary tumor is induced on a daisy stem by inoculation, deepseated secondary tumors developed from tumor strands often arise in the leaves, and these tumors convert the unilateral leaf or some portion of it into the concentric closed structure of a stem, that is into a rough and irregular growth which is crudely a stem (Pl. XI, figs. 44 and 45 and Pl. XII, figs. 46, 47, and 48), but is less compactly and perfectly formed than a true stem, less well vascularized and lignified, destitute of cork and pith, and subject to early decay. The tendency of the secondary tumors to form whorled closed structures is so strong that we often see little stemlike whorls within the big one (Pl. XII, center of fig. 48 and for details Pl. VIII, figs. 25 and 27). On the contrary, tumors occurring naturally and primarily in leaves or produced in leaves by direct inoculation are formed, of course, wholly out of local leaf tissues and in these I have not observed stem structure (Pl. XII, fig. 50) Inoculations into the upper part of sugar beet roots gave in one instance a secondary tumor on a leaf stalk which showed the ringed structure of the upper part of the root (Pl. XII, fig. 49). This is the only secondary tumor I have seen on the beet.

### VI. PHYSIOLOGICAL EFFECT OF THE TUMORS ON THE PLANT

Crown gall is usually a slow disease, at least as regards its effect on the plant as a whole. Frequently it does not kill the entire plant, and, when it does do so, several years usually intervene between its inception and the death of the plant. This question, therefore, arises at the outset. In its effect on the plant is this disease in any way comparable to cancer in man and the lower animals? Or is it only a kind of wart of no especial significance? This latter inquiry has been answered both "Yes" and "No" by plant pathologists. In other words, there has been no agreement among them. Especially have commercially interested persons tried to make out that the disease is one of no vital consequence. I have given much thought to the subject and my conclusion is as follows. A wart is a super-

ficial growth not seriously disturbing the general economy of the body. Crown gall is often a deep-seated growth, destructive of contiguous tissues. It frequently kills roots or branches on which it is located, and if it happens to be situated near a vital organ such as the growing point of a sugar beet it kills the whole plant (Pls. VII, VIIa). On certain species even when it is located on the main shoot far below the growing top it may kill the whole plant in three or four years. European grapes, peaches, almonds, raspberries, roses and willows are seriously injured by it and frequently killed. The different species of plants on which it occurs naturally, or can be induced by inoculation, vary greatly in their susceptibility to injury. We have reproduced it by pure-culture inoculations on more than 30 species of plants belonging to many different families (having now made more than 3000 inoculations) but some species apparently are not subject to it, e.g., the onion. Monocotyledons in general are more resistant than dicotyledons. In fact, as the result of our bacterial inoculations, I have not seen any well developed tumors on endogens, although minute ones have been produced on a number of species. In inoculated Paris daisies the disease is of long duration, dwarfing the plant and killing many branches, but usually not the entire plant. On the other hand, I have seen sugar-beets which were inoculated in the crown, i.e., in the vicinity of the growing point, develop large tumors and become reduced to a moribund condition within three or four months from the time of inoculation (Pl. VII, figs. 23 and 24, which are inoculations of different years). I also obtained the same result on sugar beets in 1916. Of ten plants inoculated on the crown two months ago only two have made a growth even approximating that of the controls. Of the others, two are dead and five are dwarfed and sickly (Pl. VIIa). All bear tumors. The controls are all fine plants. Other beets inoculated in a less vital part, that is, lower down on the root system, while developing large tumors have grown vigorously to maturity (Pl. VI, fig. 22). No consideration of this question can be adequate which does not take into account the different nature of plants and animals. I have pointed out resemblances,

and will now speak of a difference. Man and the higher animals may be regarded as physiological units. The higher plants on the contrary must be regarded as an intimately fused congeries of individuals, a sort of colony, something more like a coral reef than a vertebrate or at least a less centralized and definite unity than the latter. The circulation on one side of a tree is largely independent of that on the other side. The dicotyledenous tree may be split longitudinally and grown as two separate individuals. It may be cut into a thousand pieces and each propagated as a separate existence. Therefore, if cancer occurs in plants we should expect to find its action more localized than in case of animals which have a highly developed nervous system, many specialized organs, and a rapid blood stream centering in a heart. Moreover, since the embryonic layers and mature centers of growth are different in plants and animals, and since the latter are more numerous in the higher animals than they are in plants, in case of the occurrence of cancer in plants we should not expect it to conform exactly to any special type found in animals, such as glandular cancer (carcinoma), skin cancer (epithelioma) or connective tissue cancer (sarcoma), but only to show approximate resemblances, i.e., certain growth differences depending on whether it originates in the growing point, in the deeper cambium zone of the stem or root, or in a more superficial reproductive zone. And we do find certain striking differences.

Moreover, to come back to animal cancer, while the final stage of cancer is often rapid, the beginnings are frequently slow, carcinomatous nodules in the breast sometimes remaining stationary for months and even years. Epitheliomatous growths also are often slow in their development. There is not, therefore, either in its onset or in its effect on plants, any fundamental objection to regarding crown gall as cancer. A valid objection must be an histological one, or at least must be otherwise based than on the results of the disease in plants. Certainly, crown galls can not be regarded as warts, because they are not restricted to the epidermis but are destructive of the deeper tissues, and are also histologically quite unlike warts in that the deeper tu-

mors are freely vascularized and develop secondary tumors from tumor strands, like breast carcinoma and several other well-known types of cancer.

## VII. VARIOUS TYPES OF CROWN GALL

The structure of this tumor varies according to the part of the plant inoculated. We have found certain developed tissues in which we have not been able to produce tumors by our inoculations, e.g., the pith of certain plants.

*Sarcoma.* In certain ways the ordinary crown galls (e.g., Pl. I, figs. 1, 2, and Pl. VI, figs. 21 and 22) are much like sarcoma in that they involve connective tissue, develop most vigorously on young plants, or on very well nourished, actively vegetating parts of older plants, and produce large perishable tumors, many of the vessels in which are reduced to their primitive elements, and have very thin walls. I regard them as the nearest approach to sarcoma possible in plants.

Sometimes, as in case of inoculations into sunflower disks, there is very little external indication of disease, the whole or the greater part of large tumors of this type developing internally (Pl. III, fig. 7, and Pl. IV, fig. 16). In most cases the tumor, while deep-seated, ruptures to the surface early and develops in plain sight. Death of parts of the tumor appears to be due to imperfect vascularization; death of parts beyond the tumor to atrophy and cachexia. The latter seemed to be the case in some of the infected dwarfed sugar-beets to which I have already referred (Pls. VII and VII*a*). All or most of the crown galls hitherto figured in the literature are of this type.

*Carcinoma and epithelioma.* Numerous glands occur in plants and they are widely distributed but usually they are very perishable, as in the flower, or else composed of only a very few cells so that at the present time I do not know what will happen when a gland is inoculated and proliferates, but when leaf axils of Ricinus are inoculated and a tumor develops the neighboring glands of the leaf stalk (an inch or two away) are frequently invaded after a few weeks and are greatly enlarged, often to 100

times their normal size. Apparently what happens when the epidermis proliferates is shown on Plate XVII, figure 62, and this strikingly suggests what takes place in the epithelium of a mucous membrane in early stages of carcinoma, or of those downgrowths from the skin which occur in epithelioma.

One of the most interesting features of figure 58 is at *E*, where three small blastomous surface growths occur. At first sight they were taken for very rudimentary leaf buds but further study shows that although they are provided with an epidermis bearing trichomes (hairs) their cell structures is not that of any other surface organ, even in its youngest stages. In some cases the trichomes have become many-celled and blastomous. In places the epidermis is no longer distinguishable as such, and in general it has lost its cylindrical form, the cells becoming nearly isodiametric and staining unlike those of normal epidermis. The tissue below it is like it structurally and seems to have arisen from it rather than from the deeper tissues. The tissue under these tumors shows curious phenomena of erosion and there is an attempt on the part of the plant to form a cork layer under them. I believe we have here to do with tumors derived from the epidermis, i.e., with an epithelioma. Indeed, in several places bordering on this region the one-layered epidermis (equivalent to a columnar epithelium) may be seen in the act of breaking up into a several celled, deep staining, large nucleate tissue, the cells of which are isodiametric and have lost a part of their polarity (Pl. XVI, figs. 60, 61, and Pl. XVII, fig. 62). This conclusion, however, is based on only a single set of slides.

Something very suggestive occurs also in a superficial (cortical) internodal tumor obtained on tobacco in 1907 but only recently studied critically. Here the outer part of the rapidly proliferating tumor is composed of cells unlike those occurring in the deeper parts of the tumor, and suggestive of epitheliomata (See Pl. I, fig. 3, at *S* and *E*, for orientation, and for details Pl. XXIII, figs. 77 and 78). In this superficial tumor I believe there are two distinct types of tumor cells, one type derived from the cortex cells and the other from the epidermis; but from these

particular sections I can not be certain of this, because the development of the tumor is too far advanced. I have, therefore, recently made additional shallow inoculations on tobacco stems, and have obtained small tumors, from which sections have been cut, but no such phenomenon is visible in them, only one type of tumor-cell being present. To be certain, therefore, I must try, as in the geranium, the result of very shallow inoculations, wounding only the epidermal layer of cells.

*Embryomata.* We come now to embryomas. The tumors (whether primary or secondary) which have engaged so much of my time and attention for the last twelve years and which are illustrated in the preceding pages, are incapable of producing shoots. On the trunk of an orange tree in Florida I once saw a hard tumor of considerable size and unknown etiology, which was thickly set with buds, a thousand perhaps in all; and bud-bearing tumors also occur on oak trees and other trees, but I had never seen anything like this in crown gall produced by inoculation, and have been inclined to attribute these budding tumors to other undetermined causes. Indeed up to the winter of 1915–1916 I do not recall that I ever saw a bud or shoot on a gall known definitely to be a crown gall. Early in our study of this disease, however, we demonstrated by cultures and by inoculations on apple, quince, and sugar-beet that a root-disease of the apple characterized by small flat tumors out of which grow tufts of fleshy roots (Pl. XIV, fig. 53), and which was supposed to be a disease of non-parasitic origin, was actually due to bacteria scarcely distinguishable morphologically and culturally from those causing the ordinary crown gall. I was then rather inclined to assume complete identity of the organisms and to ascribe the difference in the phenomena, i.e., plain galls, or root-bearing galls, to fundamental differences in the tissues which happened to become infected, since the parasitic bacteria were not found in the new roots but only in the flat and often scanty tumor from which they originated (Phytopathology, February, 1911, vol. i, p. 10, and U. S. Dept. of Agriculture, Bureau of Plant Industry, Bulletin No. 213, p. 157). Clusters of roots were also obtained on the stem of a

collard plant (Pl. XIV, fig. 54) which was inoculated with the crown-gall organism plated from a tumor on poplar, which tumor did not bear roots. To this extent only had I seen formed organs develop out of the experimentally produced tumor tissue. Now I have seen much more, to wit, imperfect and fugitive leaf-buds and flower buds also developing from crown galls produced by pure-culture inoculations, using the hop strain of *Bacterium tumefaciens*.[1] These were first observed January 8, 1916, in shoots of red-flowered Pelargonium inoculated in the growing point, five at least out of six plants showing the phenomenon (Pl. XIV, fig. 55, and Pl. XV, figs. 56 and 57). These plants were not inoculated with this end in view but were routine checks on other inoculations. One of these tumors (fig. 57) showed one green shoot and 12 red-tinted outgrowths, assumed on account of their color to be of floral origin and shown to be such later by serial sections. Another tumor (Pl. XIV, fig. 55) showed 40 diminutive leaf buds. Had the latter been uninoculated, the bud-center although normally situated would have remained dormant. Becoming infected its cells were torn apart by the rapid growth of the tumor and stimulated to develop into rudimentary leafy organs distributed all over the surface of a part of the tumor, and growing out of it. More recently by bacterial inoculation restricted to the region of dormant buds in leaf axils, I have obtained the same phenomena on tomato and tobacco plants[2] (Pl. XIX), on orange trees (Pl. XIV), on castor oil plants, and again on Pelargoniums.

[1] These facts were discovered immediately following the Second Pan American Scientific Congress and, exclusive of a brief note in Science (March 10, p. 348) and in Jour. Am. Med. Assoc. (March 11, p. 833), were first presented in an address before the National Academy of Sciences in Washington, April 18, 1916, by permission of the Secretary of Agriculture.

[2] Also, since the above was written, on one tobacco plant by direct inoculation, i.e., without the intervention of a tumor-strand I *caused a shoot to develop out of place*, i.e., *below a leaf rather than from the axil above it*—an unheard of phenomenon! I made eight deep needle pricks on the internode directly under the leaf and about an inch below its union with the stem, introducing *Bacterium tumefaciens*. Seven of these punctures yielded small tumors of the ordinary sort. From the eighth (uppermost) puncture a shoot developed, bearing six tiny leaves (Pl. XIX, fig. 71).

After this, it is impossible to deny that the result would have been the same, had the infected needle entered the vicinity of toti-potent "cell-rests" in any part of the plant capable of proliferation. Moreover, if a microörganism can cause the proliferation of dormant toti-potent cells in a plant, it ought to be able to do it in an animal, in an ovary, let us say, in a testicle, in the thyroid, or wherever the embryonic teratomata occur.

It appears established by these observations and experiments that whether the crown gall shall develop as an embryonic teratoma, i.e., a tumor capable of developing more or less atypical blastomous tissue intermingled with a jumbled mass of young roots and shoots, or as a tumor incapable of producing organs, or even well-formed tissues, depends on what tissues of the plant are inoculated. If root or shoot or flower anlage are infected by the bacteria, then we may expect teratomata, but otherwise only an irregular tumor composed of vessels (trachei and sieve tubes, corresponding roughly to blood vessels and lymph channels), connective tissue cells, and rapidly dividing, unripe blastomous cells, but which can not give rise either to roots or shoots because not derived from toti-potent cells. The importance of this discovery lies in the fact that it correlates the most diverse forms of neoplasm, and removes one potent objection to regarding crown gall as a cancer, since it is now possible to explain all the leading types of cancerous growth by means of it.[3]

Further study of the Pelargonium tumors has proved very interesting. Figure 58 (Pl. XVI) shows a vertical section through the embryoma shown in figure 55. It is all a tumor, that is an overgrowth, but only a portion of it is blastomous

[3] This is, I believe, the first time that any one has produced embryonal teratomata! Concerning their experimental production Askanazy in 1908 made the following remarks (Verhandl. d.d. Path. Ges., Jahrg. 1907, Jena, 1908, p. 81):

Das erstrebenswerteste Resultat, ein Teratoma embryonale, ein Blastom zu erzeugen, ist bisher noch niemandem gelungen. Es fragt sich nun, nach dem ich mit Leichtigkeit Teratoide an Ratten erzeugen konnte, ob es gelingt, in dieser Richtung weiterzukommen. Alle Versuche, die sich eine solche Aufgabe stellen, sind zu fördern, da gerade unsere Unfähigkeit, experimentelle Blastome hervorzurufen, den wundesten Punkt in allen Diskussionen über die Aetiologie der Geschwülste darstellt."

(cancerous). It still shows quite clearly the various regions of the stem, e.g., pith (*P*), xylem (*Xy*) and cortex (*Cor*). Probably none of it is entirely normal, but structurally a considerable part of it approximates normal. Nearly all of the xylem (woody portion) is blastomous. The pith differs chiefly from normal pith in having scattered tracheids in it. The cortex for the most part, especially where it bears the diminutive, distorted, perishable, leafy outgrowths is covered by a normal looking epidermis. The subepidermal tissues also appear to be normal, and many of the cells in this region are full of starch. In three places, however, the blastomous portion has invaded the cortex and reached the surface (*B*, *B*, *B'*, *B''*). By the same lettering I have also indicated various interior blastomous parts. On the right side at *X* is a very interesting small strand of blastomous tissue, only a little thicker than it is wide, which arises in the cambium and passes outward between the coarse cells of the cortex into a superficial blastomous area (*B'*) smaller and less compact and presumably younger than the cambial one at *C*. Soon after leaving the blastomous cambial region this strand gives off a branch which passes into the superficial blastomous region *B''*, which also, I think, is of more recent origin than *C*. All these details are shown clearly in the serial sections and such of them as are visible in this section are shown in detail in figure 59. This strand is made up of small, deep-staining, embryonic, atypical, blastomous cells entirely surrounded on all sides by the large, feebly-staining, normal looking cortex cells. I am not entirely clear as to the nature of the two superficial small dark bodies in this region. They are composed of normally arranged and normal looking young cells filled with deep-staining protoplasm, and are probably non-blastomous leaf or flower anlage.

*Secondary teratomatous tumors.* In man the embryonic teratomata metastasize freely and the secondary tumors may either repeat in full the structure of the primary tumor, or may leave behind most of the teratoid elements, or, finally, may leave behind all of the latter, growing in the daughter tumors only as a sarcoma, or only as a glioma (Askanazy).

As soon as I had discovered the teratomata on the Pelargonium the query arose: Will they develop secondary tumors? Up to this time (three months) they have not done so. The infected tobaccos, however, have given striking results. The first ones were inoculated by needle pricks in the upper leaf axils on January 19 and in less than a month (February 14) they yielded numerous well-developed small axillary tumors covered with diminutive shoots bearing dwarfed leaves. Not only did these peculiar tumors develop in the leaf axils (where the plants were inoculated) *but also at a distance, and in both stems and leaves*, i.e., tumor-strands developed from the base of the primary teratomatous tumors passing upward through the soft stems (region of the cortex) and outward into the young leaves following in the petiole sometimes the inner axis of the leaf arch as in the daisy (fig. 74), and sometimes the outer axis (fig. 69). Out of these strands tumors have developed and ruptured their way to the surface. These secondary tumors occur numerously on shoots, petioles and leaf-blades, and, *they are covered with shoots bearing tiny green leaves.*

In one plant which was inoculated in the very immature top, before the internodes had elongated and while the leaves were yet small, the tumor-strand on February 14 was traced a distance of 10 inches, entirely in the cortex, from which at intervals it sent to the surface leafy tumors interspersed with non-leafy ones, and ended in the midrib (the under surface of the leaf) in a small tumor which bore a green leaf bud (fig. 69 at *X*). Some of these tumor strands in the tobacco are large enough to be plainly visible on cross-section under a hand-lens, and are even close enough to the surface to show through, as may be seen in figs. 67–69, while others require the compound microscope. Yet they are invariably present, and, again, as in human cancer, the structure of the secondary tumor repeats that of the primary tumor, but in this instance that which is repeated is an embryonic teratoma. These results are illustrated on Plates XIX, XX, XXI, and XXII.

Probably there still exists in the minds of many a doubt as to the invasive character of the tumor tissue in crown

gall. This doubt is based, I suppose, on the belief that the neoplastic tissue may represent a reaction of the local tissues. This objection must be answered in case of the teratomatous (embryonic) secondary tumors. Here we have organs introduced that are foreign to the leaf, and the anlage of which we know to be definitely located in the stem, to wit, in the leaf axils. There can be no question in this case that the tumor-strand is an invasion, nor can there be much doubt that it has dislodged from the stem, and carried along with it into the leaf, fragments of the axillary shoot anlage which later have developed into leafy organs in the secondary tumors. The only alternative is to suppose that the tobacco leaf, while it never normally develops shoots, has in it widely distributed the potency of shoot development, i.e., many groups of embryonic toti-potent cells, awaiting only approach of the necessary stimulus to grow into organs. This would mean that the shoots on the secondary leafy tumors which I have figured might be either derivatives from the stem tumors by way of a tumor-strand (which can be seen connecting them with the primary tumor) or might arise locally from toti-potent cells stimulated into growth by the presence of the tumor-strand. There is nothing to oppose this second view, except the before-mentioned fact that in nature we do not find shoots growing out of tobacco leaves (see Pl. XXII, fig. 76 at *S*); and in favor of it there is the fact that some plants (Gloxinias, Begonias, Sempervivums, etc.) develop roots and buds from leaves under favorable circumstances i.e., when cut and kept on or in moist earth or even in moist air. The leaves of the West Indian *Bryophyllum calycinum* do this even while on the plant. But these plants have been supposed to be exceptions to the general rule.

*Atypical teratoid tumors directly from leaves.* To determine the matter experimentally for tobacco I made nearly 1000 needle-prick inoculations on 43 young upper leaves on 16 shoots of 8 individuals from the same lot of tobacco plants as those shown in figure 66. From these inoculations I obtained a hundred small tumors about forty of which developed shoots (Pl. XXII, fig. 76*a*). None of these leaves were inoculated in the axillary

part, but most of them in the mid-rib at some distance from the axil. I made, also, 150 cuttings from the tobacco leaves (midribs) none of which rooted or developed shoots.

We must conclude, therefore, that the blastomous parts of the secondary shoot-bearing tumors in the tobacco leaves are derivatives from the shoot-bearing axillary stem tumors, *and must be what they appear to be, namely, invasions from the stem*, and that the teratoid elements are developed either from toti-potent cells torn loose from the axillary anlage and carried along with the tumor-strand into other internodes and into leaves, or that they are formed directly out of local leaf tissue, i.e., from groups of toti-potent leaf cells, which, but for the bacterial stimulus brought to their vicinity by the tumor-strand, would have remained dormant. Clearly both processes might conceivably occur.

*Shallow stem inoculations.* Something may now be said on the result of deep *vs.* shallow stem inoculations in areas free from toti-potent cells.

If inoculations are made into the cambium (see Pl. X, fig. 35, line *C*) of the internodal region on young growing shoots, in regions free from toti-potent cells, deep-seated persistent tumors arise (Pl. IV, fig. 17) which retain some of the tissue-forming powers of the cambium. Elements of both wood and bark are found in them, but so far as I have observed the tumors have no power to organize these elements into organs, such as true stems, leaves or flowers, or buds capable of developing into such organs. They are not then embryomata. Certain plant tumors may be covered all over with buds, but not crown galls of this type. At most, roots may develop out of certain of these tumors when planted, but only, I believe, when root-anlage have been stimulated. The cells, then, of the deep-seated crown gall have lost a part of their coördinating power, and many of them have become unripe, vegetating cells.

If, on the contrary, very superficial inoculations are made on the stems of plants, i.e., into young cortex before cork has formed on it, superficial hyperplasias of limited growth arise. These tumors involve neither wood, cambium nor bast (phloem). They are formed (unless it may be that epidermal cells are also

sometimes involved) wholly out of cortex cells, which under the influence of the bacteria begin to grow rapidly, become much smaller and more embryonic in their nature than the surrounding cells, and are then able to vascularize themselves to a limited extent (Pl. I, fig. 4), although such vessels (trachei) do not occur naturally in the cortex. This shows incontestably, I think, that the inoculation has converted the ripe cortex cells, or cells which normally would have become ripe cortex cells, into a more embryonic tissue. The trachei also give striking evidence of this, because here under a changed stimulus a cell mother of one kind has given rise to a cell of another kind. This is what I suggested as possible in 1911 (Bull. 213, p. 171) without at that time having the evidence to support it. Unlike that of the deep-seated tumors, this spurious stroma has no connection with the general vascular system of the plant and is, therefore, of small service to the tumor. The inoculations which yielded these tumors were made with a needle projecting only $\frac{1}{3}$ mm. beyond a broad base so that under no circumstances could the wounds reach into the cambium or even into the deeper tissues of the bark, and moreover the serial sections show that these tumors are confined wholly to the outer part of the cortex. The inoculations were made into cells of the outer cortex like those seen in the outer part of Pl. X (fig. 35 *Cor.*) and the result is shown in Plate I, figure 4 and Plate V, figure 19. Similar results have been obtained on tobacco (Pl. I, fig. 3).

### VIII. ELEMENTS OF THE PRIMARY AND SECONDARY TUMORS

The histology of the primary (stem) and secondary (leaf) tumors in the Paris daisy appears to be the same. Some details remain to be worked out but it is not likely that any future discoveries will greatly modify the general conclusions as here stated. In the primary tumor of the Paris daisy, such as that shown in Plate IV, figure 17, in addition to the tumor cells which may arise, as we have seen, both from the cortex (Pl. I), and from the deeper true cambium, there is a supporting stroma which in all the large tumors is an ingrowth from the stem.

This stroma consists both of cells and of vessels. The cells are the connective tissue cells of the stem, i.e., medullary ray-cells from the wood and the inner bark, and cortex cells from the outer bark as shown at *X* in Plate IV, figure 17. These cells may be unmodified and external to the tumor proper (blastomous part), may be surrounded and crushed by it as shown at *C* in Plate XII, figure 48, or may be stimulated into repeated division (Pl. I, fig. 4, below *X* and Pl. XXIII, fig. 78); whether in that case they are also infected, or are only acted upon from a distance, is not known. The vessels of the tumor consist of trachei and of sieve tubes. The trachei are the ordinary pitted aerating and water conducting vessels of the stem, which here are always separated into smaller groups than in the normal wood, often into their individual elements, and are twisted and distorted into the most bizarre forms by the growth of the tumor, their polarity being greatly disturbed. As we have seen, these trachei may also be formed under the influence of the bacterial stimulus in the outer bark out of cortex cells, but only to a limited extent. Under these circumstances they do not functionate, at any rate not as water carriers, as is evidenced by the limited growth of these superficial tumors and by the failure of these trachei to anastomose with the deeper trachei, as shown by serial sections. Even in these superficial stem tumors there appears to be an effort on the part of the plant to orient the vessels as in a stem, as shown by the structure. In the normal stem the trachei are laid down on the inner side of the cambium to form one of the elements of a compact cylinder of wood (Pl. X, fig. 35 *Xy*). The sieve tubes (Pl. X, fig. 35, *Ph*) which are the slime-conducting vessels of the inner bark, carriers of the elaborated proteids, form the other vessel element of the tumor, equally separated, twisted and distorted. The carbohydrate elements of growth, starch and sugar, are chiefly stored in and moved through the connective tissue cells (pith, medullary rays of the wood and inner bark and cortex cells of the outer bark). No starch is stored in actively dividing tumor cells, but sometimes much is stored in stroma cells of the cortex, as in the outer parts of the Pelar-

gonium tumor shown in figure 58, in and under the proliferating buds. The stroma of the secondary tumors appears to be an ingrowth from that of the primary stem tumor by way of the tumor-strand, rather than an ingrowth from the normal tissues of the leaf at the level of the tumor. The progressive growth of the stroma along with the tumor cells is shown very clearly on Plate XIII, figure 52, taken from the sunflower. I do not yet know whether bast fibers occur in the tumors.

In the bud tumors on the geranium, surface portions of the tumor surrounding the buds appear to be quite normal, i.e., covered with a normal looking glandular epidermis, and subtended by normal cortex, the cells of which are filled with starch (Pl. XVIII, fig. 64) and fed, more or less scantily, by a normal vascular system, yet the deeper tissues are manifestly blastomous (Pl. XVIII, fig. 64 at *B*, *B*) In hairy-root of the apple one sometimes looks almost in vain for signs of a tumor under the tuft of roots, and yet even in such cases the bacterial stimulus is present. How restricted are the root-anlage of stems, or rather how sharp is their boundary is well illustrated in the collard stem shown in Plate XIV, figure 54. Here the lower half of the lower tumor is covered with diminutive roots corresponding to an infected root-center, while the upper half of the tumor is entirely free from roots and may be supposed to have developed from neighboring tissues not endowed with the power to form roots either when planted in the earth or when stimulated by a tumor-forming microörganism. Had this stem not been inoculated the root anlage would have remained dormant. Further, the infected needle, which entered a little above the site of this tumor, caused the development of a rootless or nearly rootless tumor.

Whether sieve tubes develop in the superficial bark tumors of the daisy along with the trachei is a matter not yet determined. Later: They are present.

Spiral vessels occur in the inner wood of the Paris daisy (Pl. X, fig. 38), but have not been seen in the tumors except as accidental inclusions, torn away from their original site and carried sometimes long distances by the energetic development of the

tumor. What would happen when tissues are inoculated which contain only vessels of two types (sieve tubes and spirals) as in case of cucumber leaves, remains to be seen. One would suppose that spiral vessels must then enter into the normal growth of the tumor. Later: These leaf inoculations developed small tumors containing trachei, i.e., vessels not normally present in cucumber leaves but natural to the stem, which we may suppose to be a more primitive structure than the leaf.

Earlier, some reference has been made to the occurrence of whorls of trachei in the middle of secondary tumors in daisy leaves, thus giving the appearance of diminutive stems within a larger stem. This phenomenon is seen still more strikingly in cross-sections of the tobacco stem shown on Plate XIX, figure 68, where under the influence of the tumor-producing bacteria a small stem-like structure, (the tumor-strand) has made its appearance between the coarse outer cells of the cortex. This stem within a stem (Pl. XX) consists of a closed cylinder of wood wedges, spirals, trachei, connective tissue, and (?) pith, surrounded by a cylinder of cambium outside of which is a cylinder of phloem (Pl. XXI, fig. 75). Cortex and epidermis are wanting and are not needed for protection because the surrounding normal cortex of the stem serves that purpose. A more unexpected and astonishing pathological phenomenon I have not seen. It is like finding a set of teeth in a tumor of the brain. This little stem within a stem is derived from an axillary embryoma and gives rise above to the leafy and non-leafy tumors I have figured. Almost or quite all its visible elements at this level are teratoid, rather than blastomous, yet it had the power of developing blastomous tumors as shown by the growths arising from it and rupturing through to the surface of the plant.

Plate XXII, figure 76, from the same series as figure 67 but at the end of 40 days, shows the same phenomena further advanced; all the numerous small shoots are from tumors and all are abnormal. Special attention is called to the one marked (*S*), growing from the midrib of a leaf (*X*) without visible external signs of blastomous tissue except an internal thickening on one side near the base. It is weird as an epignathus or a congenital sacral teratoma.

The surface of the common daisy tumors is brown and suggestive of a cork layer, but so far as examined they appear to be naked and unprotected by cork, nor are the teratoids I have produced enclosed in a cyst.

## IX. SYMBIOSIS

One has, I think, made out a symbiosis if he can prove that some tissues of a particular organism benefit by the presence of the microörganism, whether or not all tissues do so. A symbiosis in which there is equal and impartial giving and taking must be very rare in nature. I do not know of any such. Usually one symbiont gets the lion's share. In lichens the algal cells are imprisoned, and the fungus appears to receive the greater benefit. In the bacterial root nodules of legumes, if one considers only the parts attacked, one cannot speak of a symbiosis because the host tissues are occupied and destroyed, but if from the bacteria the plant as a whole receives nitrogen, which it would not otherwise get, then it is a symbiosis in spite of the local destruction of tissues.

In crown gall on the Paris daisy, and also on other plants, one of the striking things both in the proliferating cells of the tumor and in the deep tumor-strands, when these are large enough to be seen readily, as in Pl. X, fig. 36, is a green stain due to the presence of great numbers of chlorophyll bodies. Why are these present in such numbers, especially in the deeper parts? We know that the bacteria must receive both sugar and proteids from the host plant, otherwise they could not grow, and must give off, as the result of this growth, ammonia and carbon dioxide, which are plant foods. If now the chloroplasts make use of either of these substances, as seems likely from their great abundance, then there is a true symbiosis, a give and take, no matter what happens to the plant as a whole. The mild behavior of the crown-gall bacteria within the parasitized cells, as compared for example with what goes on in tuberculosis and leprosy, or in plant cells occupied by the root-tubercle organism of legumes where the cell is stretched, the protoplasm consumed, and the nucleus destroyed, by the ever-increasing

swarm of the bacteria, also points to symbiosis as a reasonable hypothesis, to say the least. And to state that symbiosis never causes cell proliferation is to forget what takes place in lichens where the algal cells proliferate very freely under the stimulus of the fungus. It is to forget also what takes place in keffir and its allies.

## X. NATURE OF THE ORGANISM CAUSING THIS TUMOR

The organism causing this plant tumor is a feeble cell-parasite. It is a schizomycete which we have named *Bacterium tumefaciens*. It is introduced through wounds, and multiplies within the cells rather than between them or in the vessels. There are no abscess cavities. It does not disintegrate the tissues or kill the cells which it occupies, but urges them into repeated and hasty division. It multiplies within the parasitized cells only in small numbers which are hard to see either in fresh material or stained. The organism does not occur either in the vessels or in the intercellular spaces except possibly sometimes near the needle wounds in primary inoculations. Here at times in stained sections a slight multiplication of some schizomycete can be seen to have taken place, but whether it is this one or some other is unknown, with the chances in favor of its being an intruder. Ordinarily, in the undecayed growing tissues there are no bacteria visible in the vessels or between the cells, and no granules of any sort. The organism is of a size to be seen easily, if it were so present. It must, therefore, lie concealed within the granular contents of the cells. Moreover, I can not now say unqualifiedly that I have ever seen the organism within the cells. At times I have thought so, but I am now again in doubt. I have seen under very high powers of the microscope in fresh sections occasional rod-shaped bodies moving slowly within the cells. All attempts at differential staining by means of anilin dyes have failed, in spite of repeated trials. Subsequently by means of gold chloride, we stained within certain of the tumor cells rod-shaped bodies which I interpreted, perhaps prematurely, as the bacteria. They agree in form and even show branching rods, but nevertheless may be normal organs

of the cell. For the present then we have to depend on culture methods and on diffusion methods for demonstration of the presence of the bacteria in the tumors. They can be cultivated out of the tumors when not too old by means of agar-poured plates, although usually the inoculations must be heavy and generally a week or more must be allowed for the bacteria to develop. The reason for this is the fact that inside the cells the organism readily passes over into Y-shaped and variously branched forms, which may be obtained by diffusion out of the cut cells in water on slides. These are either dead, or nearly dead, and then only slowly to be coaxed back into their normal form and activity in suitable media, such as nutrient agar-agar, or in the new conditions arising within the daughter cells at the time of cell division. The writer believes that the stimulus to this abnormal cell division is a bacterial endotoxin which can become active on the cell nucleus of the host-plant only when the bacteria are dead and their membranes have thus become permeable, but how it takes place is a matter to be worked out experimentally. The numbing and killing substance is conceived to be an acid by-product of bacterial growth within the parasitized cells. *Bacterium tumefaciens* produces an inhibiting acid from grape sugar in flask cultures and may be supposed to do the same within the cell. The organism is a small non-liquefying, non-sporiferous, white schizomycete, motile by means of polar flagella. It grows on a variety of culture media, but requires frequent transfer on most media to keep it alive. On the surface of agar-poured plates the colonies are small, circular, raised, smooth, translucent and wet-glistening. If very young unclouded peptone bouillon cultures (less than 48 hours old) are held up to the light and shaken violently by oblique downward jerks, the growing organisms can frequently be seen in the fluid in the form of faint darting bands or strings. It loses virulence slowly in the laboratory, but even when first isolated some strains are much more virulent than others. There are also slight morphological and cultural differences, and pathogenic preferences, on the part of the organism as isolated from various plants.

Furthermore, we must assume either that *Bacterium tumefaciens* is frequently accompanied by, or even supplanted by, another organism indistinguishable from it on agar-poured plates and on other media, or else that some of its own elements within the tumor have lost all pathogenic power, as shown by our inoculations, while retaining the power to grow with the proper appearance on media. From what I have seen I am inclined to the latter view without being able to explain how it comes about. It is probable, however, that under some circumstances there takes place rapidly within the tumor that which we know to take place slowly in our culture media, i.e., loss of virulence. If this is true, we have in it a means for explaining receding tumors and the recovery of the plant, and also perhaps a whole tribe of benign tumors, which, as in man, frequently grade off so indefinably into malignant tumors. In this connection I am convinced that we are on the threshold of far reaching discoveries.

## XI. ANIMAL INOCULATIONS

The killing temperature for those strains of *Bacterium tumefaciens* with which we have experimented is a little below the blood temperature of man and the higher animals, but it has seemed worthy of trial whether with them it might be possible to produce tumors in cold-blooded animals (frogs, fish, lizards). The response of susceptible plants to inoculation is so prompt and striking, that it seems, on first thought, as if it would be very easy to induce like growths in some susceptible animal. Cancers occur naturally not only in man and a great variety of the warm-blooded animals, but also in cold-blooded animals, so that the animals which were selected for inoculation seemed to be appropriate for the test. It must be confessed, however, that nothing was obtained corresponding to the striking tumors which soon appeared on the check plants (Pl. VI). In case of brook trout inoculated in the eye-socket, several times small nodular growths appeared in the eye-muscles and elsewhere, but the fish died before it could be determined whether these growths were really malignant growths. In the animal inoculations there have been two difficulties: (1) the resistance of the animal body

to the inoculated organisms, probably by means of leucocytes; (2) the early death of inoculated animals, either from septicaemia due to the inoculated cultures or from other causes. In one case we plated from the dorsal aorta of an inoculated anaemic fish a practically pure culture of *Bacterium tumefaciens*, with which we then produced well-developed tumors in plants. In this connection interest attaches to statements of recent German workers (Friedmann, Bendix, Hassal and Magnus in Zeitschrift für Hygiene und Infektionskrankheiten, April 23, 1915; and elsewhere), claiming *Bacterium tumefaciens* to be the cause of a purulent meningitis and other morbid (ulcerous) conditions in man.

### XII. RELATION, IF ANY, TO CANCER IN MAN

No claim is made that the organisms which we have isolated, and with which we can reproduce these plant tumors at will, are the cause of human cancer. On the other hand, evidence is advanced that they induce a set of phenomena which, allowing for the differences between the higher plants and animals, follow a strikingly parallel course. No broadly inclusive definition of cancer can be drawn that will not, along with the human and animal neoplasms, include also these plant tumors as true cancers. Like animal cancers these crown-gall tumors behave exactly as if the cell itself were the parasite. Indeed, Jensen in 1910 called special attention to them as likely to be of as much service in throwing light on the etiology of neoplasms as were his mouse cancer,[4] inasmuch as they could be inoculated, and were, moreover, not complicated by, or due to the presence of any organism! But such is not the case, and there lies the gist of the whole matter. The cell itself is not the parasite, as Jensen thought, because we have proved these tumors to be due to a specific microörganism, a feeble, intracellular, schizomycetous parasite, which has no power to kill the cells but only the power to set them growing. Therefore, "the cell is the parasite" only in the sense that it is urged on by a schizomycete. As to the cause of animal cancers nothing is yet definitely

[4] Jensen: Von echten Geschwülsten bei Pflanzen. Trav. 2me. Conf. Inter. p. l'Étude du Cancer (held in Oct., 1910), Paris, 1911, pp. 243-254.

proved beyond the very iconoclastic and suggestive fact brought out in recent years by Rous of the Rockefeller Institute that sarcoma in fowls is due to a filterable virus, i.e., *to something, separable from ths cell itself, which can persist after the death of the cell.* This may be regarded as having advanced the subject a great way in the direction of the contention that human and animal cancer is due to an intracellular parasite, since we know of no chemical substance, enzyme or other, capable of multiplying itself idefinitely. Only a living organism can do this.

## XIII. SUMMARY

Once more, to recapitulate, I would call attention to the growth without function exhibited by these crown gall tumors; and to the embryonic character of the proliferating tumor cells as shown by their small size, by their large nuclei, lying in thin cytoplasm, by their great affinity for stains, and by their hasty division (exceptionally tumors of the size of Pl. VI, fig. 22, some of which have measured 3 by 4 by 5 inches, may be developed in six weeks). I would call attention to the atypical arrangement of the tissues, to their loss of polarity, and to the anaplasia or undifferentiation of the cells, since they are not able to produce either normal wood or normal bark, although developed out of cambium cells which have that power; in other words, what they have gained in vegetative vigor by becoming tumor cells they have lost in the power to differentiate tissues. The non-capsulate, marginal growth, imperfect vascularization and early central necrosis; the existence of invasive strands and the occurrence of daughter tumors that reproduce the structure of the parent tumor, are features which emphasize the neoplastic character of these growths. Of interest also is the fact that by means of inoculation with the same microörganism different types of tumors varying in structure, according to the type of tissue invaded, can be produced, the most complex type containing along with the blastomous elements a jumbled more or less fused mass of embryonic organs and fragments of organs, equivalent to the foetal fragments occurring in the atypical animal teratoids.

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## PLATE I

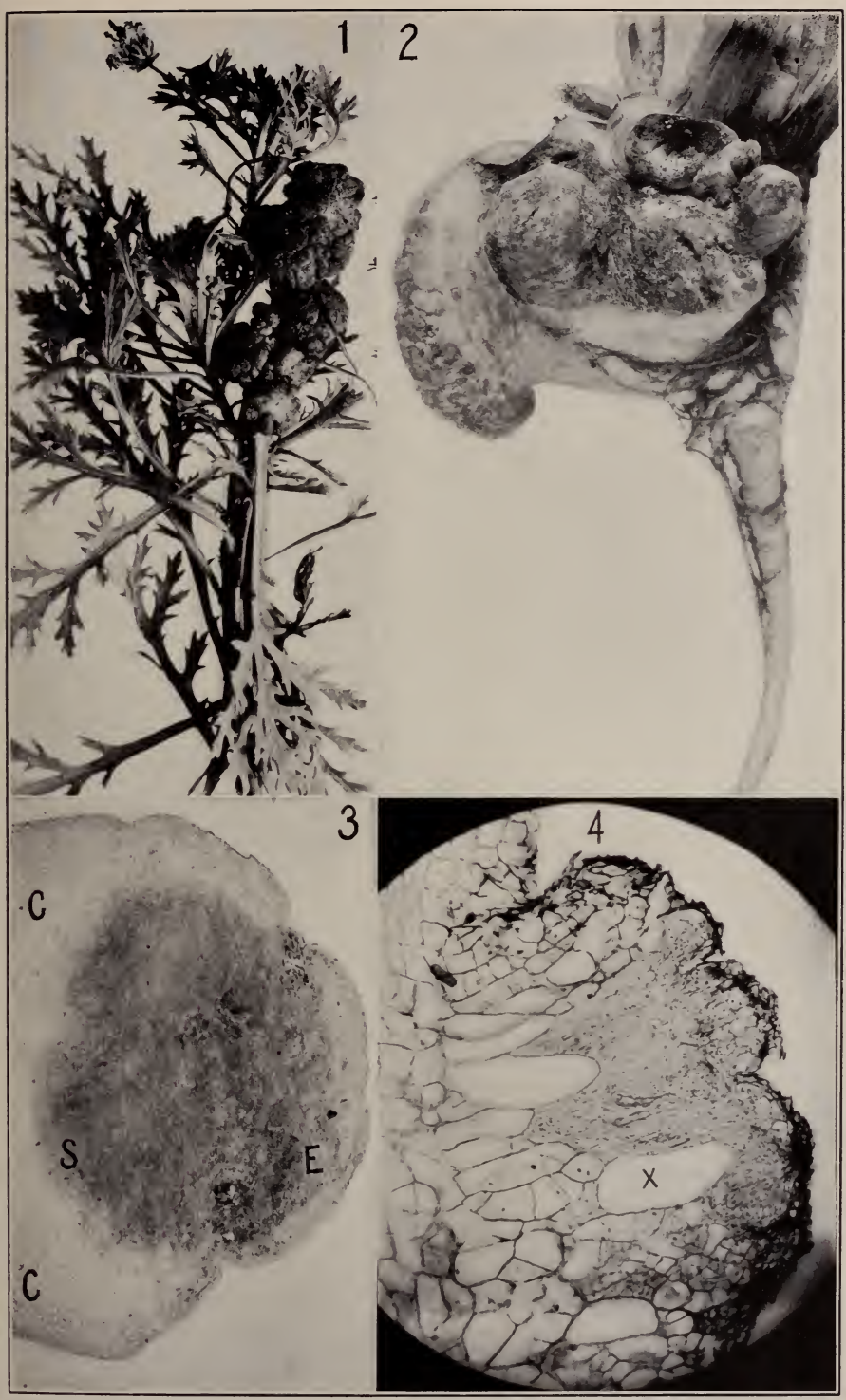
### Examples of Crown Gall on Shoot and Root. Structure of Young Primary Superficial Tumors

1. Crown gall on Paris daisy. Natural infection on upper part of the stem. The disease escaped to this plant from one of our inoculated hothouse plants. Two-thirds natural size.

2. Crown gall on sugar-beet. Pure culture inoculation of 1913, using organism from hop tumor. Necrosis just beginning (in the upper part). Time, $3\frac{1}{2}$ months. About one-half natural size.

3. Section of cortex of a tobacco stem including a young tumor produced by a needle prick introducing *Bact. tumefaciens* from the daisy. On the advancing margin, as at *S*, coarse-celled tumor tissue, i.e., normal cortex cells becoming blastomous. *C,C*, unchanged cortex. For details at *S* and *E* consult Plate XXIII.

4. Cross-section of crown gall on stem of Paris daisy, shallow inoculation, all of the fine-celled growth has been produced out of the large cortex cells. Trachei (vessels) in the tumor tissue above *X*, action at a distance below *X*, i.e., division of the large cells into smaller blastomous ones. Time, 82 days.

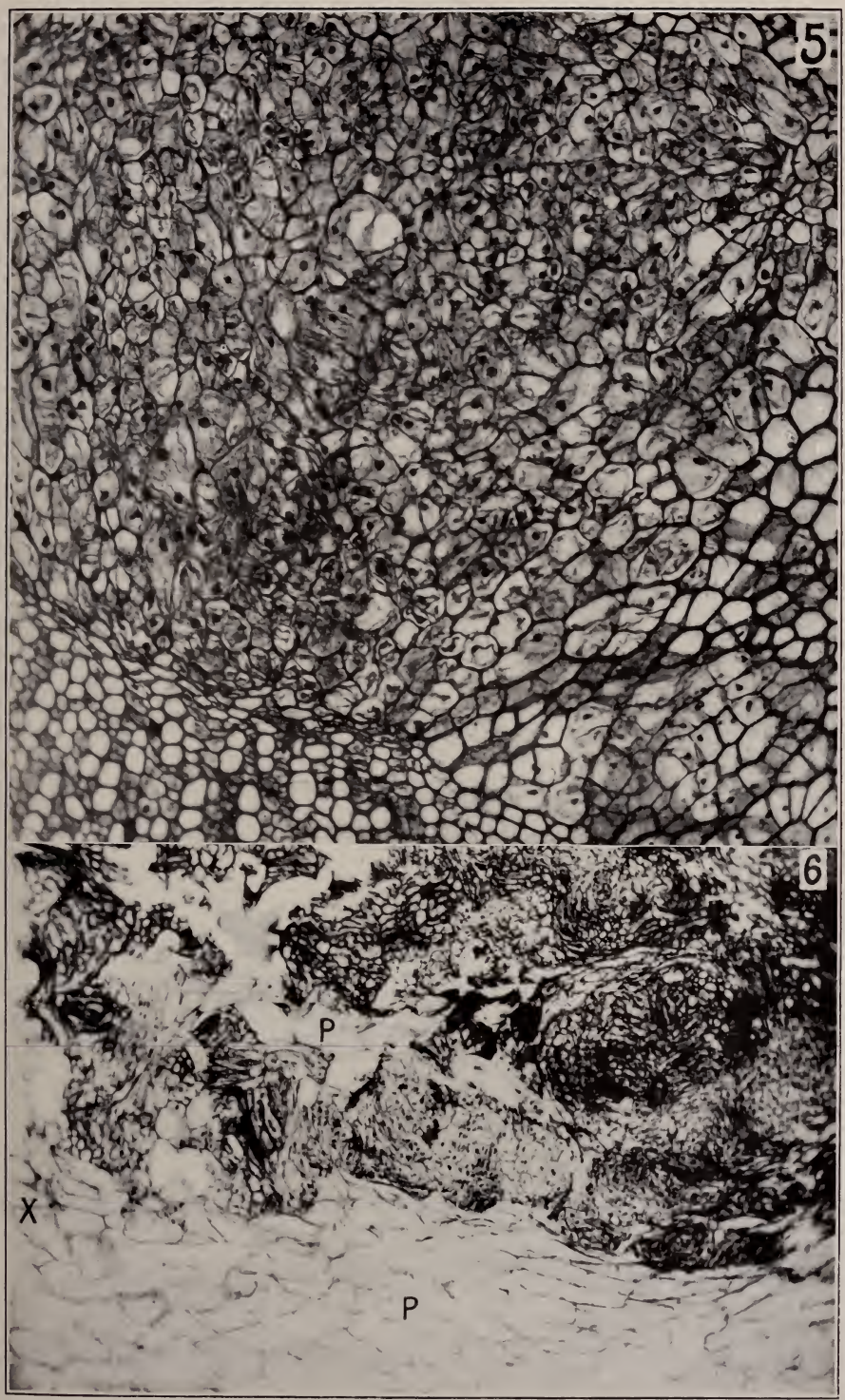

1
2
3
C
S
E
C
4
X

## PLATE II

### Embryonic Tumor Cells. Invasion of Pith

5. Crown gall on inoculated Paris daisy. Cross-section of secondary tumor in a leaf showing nearly all of the central tumor-strand which is composed of rapidly proliferating cells with large, deep-staining nuclei. Below, in the right corner, a proliferating medullary ray (wood ray). At the right and left of this are vascular portions of the tumor.

6. Crown gall in inoculated sunflower disk (back part), showing the embryonic tumor cells invading the large-celled pith (*P,P*). A detail from one of these sections made a little beyond *X* is shown on Plate XIII, figure 52.


5
6
P
X
P

## PLATE III

### Inoculations on Sunflower Disks in August, 1915, by Means of Needle Pricks, Using the Hop-Tumor Organism

7. Large tumor developed wholly within the flower disk except the part above *X* (see next fig.). *Y* corresponds to level of section shown in figure 9. The arrows indicate where some of the soft white pith surrounding the hard greenish gray tumor was removed in order to show the outlines of the tumor. The tumor ended abruptly in the pith 1 mm. below the basal section. Length of tumor, 4 inches. It was curved and is foreshortened in the photograph. Plant No. IV. Time, 6 weeks.

8. Face of disk of inoculated sunflower No. IV, bearing 19 tumors on the bracts and 7 on the disk. *X* is the one shown in vertical section in figure 7. About one-fourth natural size. Necrosis has begun at *N*.

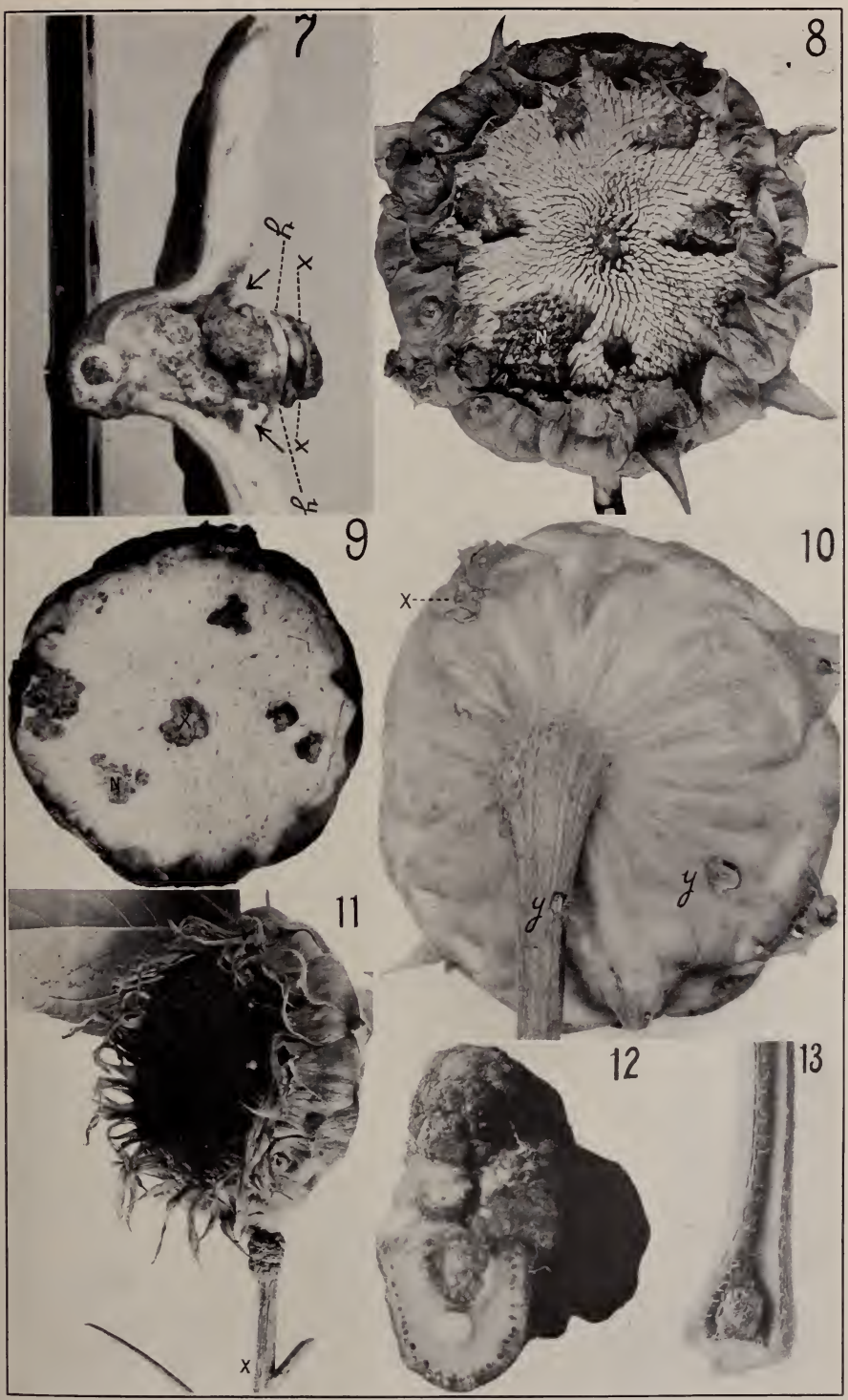
9. Horizontal section through the disk shown in figure 8, the level corresponds to *Y* in figure 7. These tumors are all downgrowths from the richly vascularized tissue which bears the seeds (level of *X* in figure 7). They lie in pith but they are not converted pith cells. The dark lines and specks in the white background are the normal vascular bundles of the pith.

10. Back of the sunflower disk shown in figure 8. *Y*, *Y* are wounds, not tumors. There is a small tumor bursting through at *X*. Surface of stem free from tumors.

11. Disk of inoculated sunflower No. 1. Slight evidence of tumors on the disk where inoculated, but in the stem there was a tumor-strand down to *X* and several deep-seated tumors are bursting through the stem above this point. Much reduced. (For back view, see fig. 14).

12. Cross-section of tumor-bearing stem shown in figure 14. The wood cylinder is enlarged and the diameter of the pith is reduced. About natural size.

13. Upper surface of petiole shown at *Y* in figure 14. Deep tumor bursting through its axil and superficially unconnected with tumor on the opposite side of the stem at the same level.


7
x
x
8
N
9
x
N
10
x
y
y
11
x
12
13

## PLATE IV

### Sunflowers Continued. Structure of Primary Tumor in Daisy

14. Back of inoculated sunflower disk No. 1 shown in figure 11. The tumor-strand in the pith extended downward a distance of 5 inches and from it several deep-seated tumors have ruptured their way to the surface of the stem. Under the swollen place *Z* is one not yet ruptured. At *T*, *T*, tumors are rupturing to the surface. *X* corresponds to the *X* in figure 11 or would were a little more of the stem shown. *Y* is the back of figure 13.

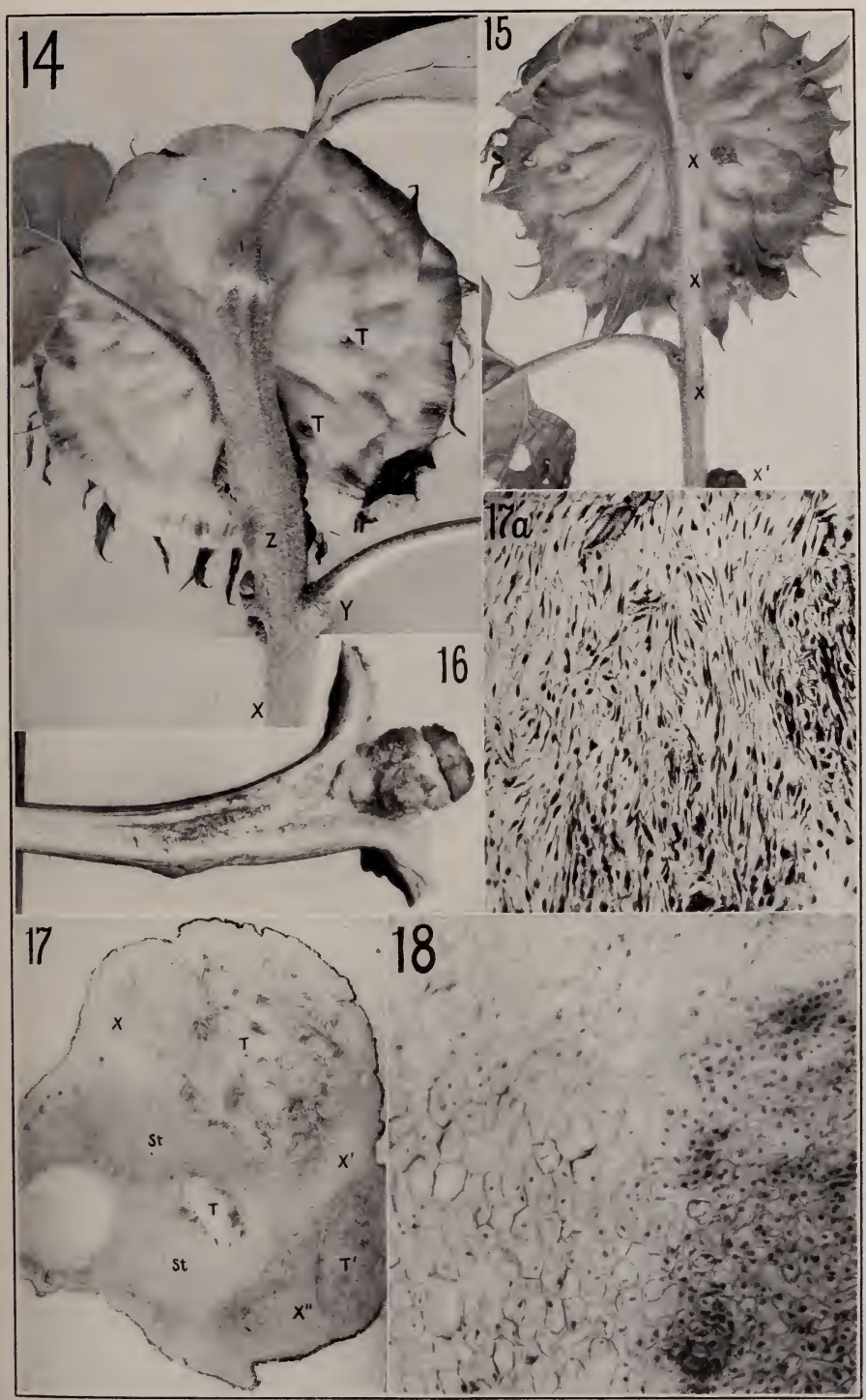
15. Back of inoculated sunflower disk No. II. A tumor-strand was traced down the stem 8 inches, i.e., to the tumor in the leaf axil (*X'*). Deep-seated small unruptured tumors at the swollen places marked *XXX*. Tumors are also bursting through the back of the disk.

16. Hidden tumor in inoculated disk of sunflower No. II. Less than one-half natural size. Time, 7 weeks.

17. Cross-section of young deep inoculated tumor on stem of Paris daisy. Bark, wood and pith of normal stem at left. On the right side an enormous enlargement of the wood cylinder, the vessels of which (*St. St.*) enter the tumor. The cortex (also enlarged) may be seen entering into the composition of the tumor at *X X' X''*. Three principal masses of feebly vascularized, rapidly proliferating blastomous tissue (*T T T*) are here visible. There is no capsule.

17*a*. Spindle shaped blastomous cells from outer portion of *T'* in figure 17.

18. A detail from figure 17 at *X* showing normal (?) cortex cells (at the left) and adjacent, small, round-celled, deep-staining embryonic tumor tissue at the right.


14
T
T
Z
Y
X
15
X
X
X
X'
16
17a
17
X
T
St
X'
T
St
T'
X"
18

## PLATE V

### Result of a Superficial Inoculation. Necrosis of Tumors

19. Cross-section of cortex of stem of Paris daisy at the level of a small tumor resulting from a superficial inoculation. The figure shows the deeper two-thirds of the tumor and the adjacent large cortex cells (*C*) out of which it has developed. Epidermis at *E*. Tumor tissue deep staining, but photographed from a faded section. Numerous trachei developed in this tumor but they have no connection with the deeper trachei of the stem. Time, 29 days.

20. Crown galls on sugar-beet. Result of pure-culture inoculations using organism from a hop tumor. Tumors larger than the beets. Necrosis well advanced. Time, 104 days. About one-half natural size.

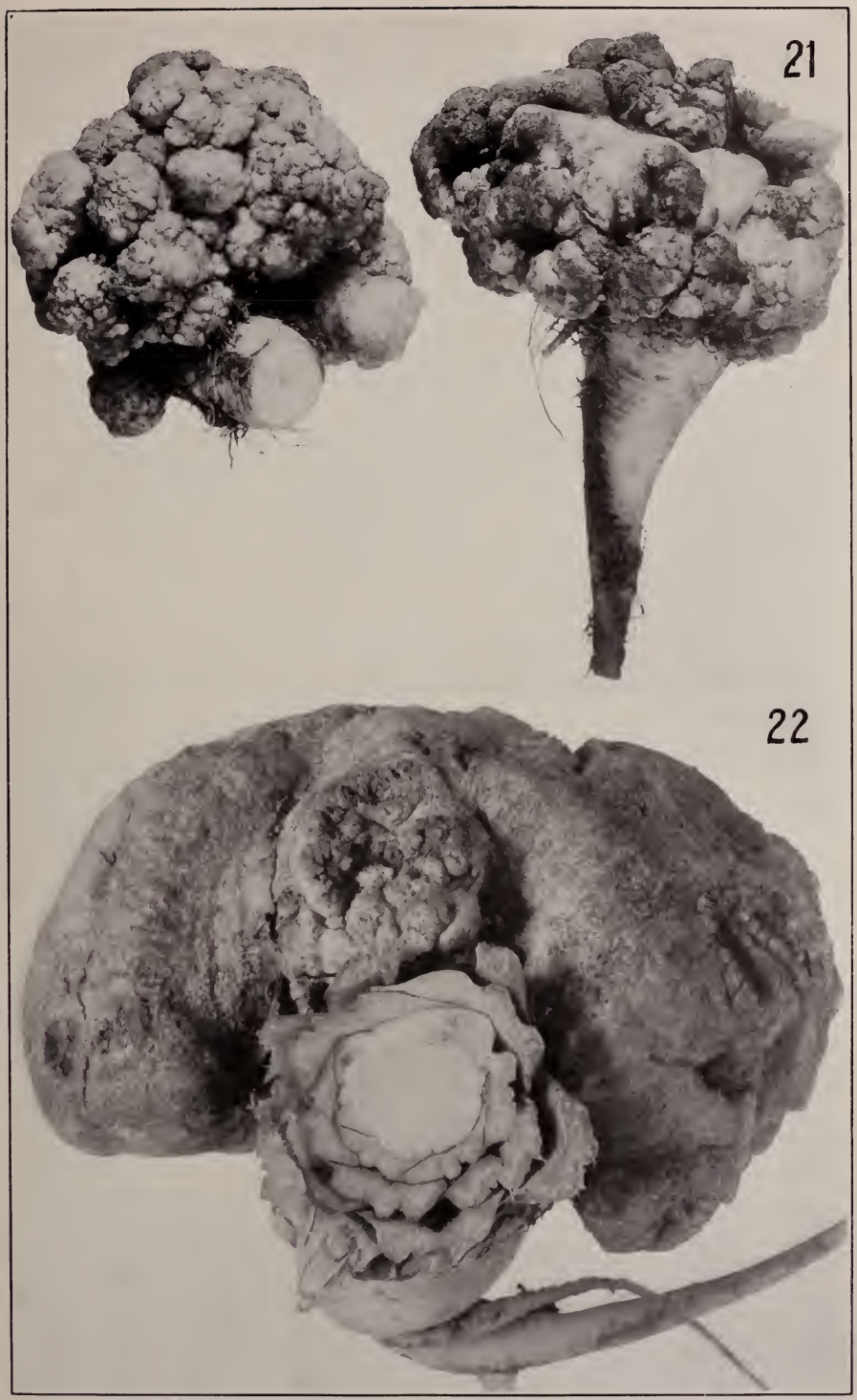

19
C
E
20

## PLATE VI

### Sarcoma-like Tumors on Sugar-Beets

21. Crown gall on sugar-beets (cauliflower growth). Result of pure-culture inoculations using organism plated from a tumor on poplar (Populus). About two-thirds natural size. Left root viewed from below. Necrosis has begun in tumor on the right. Time 82 days.

22. Crown gall on sugar-beet photographed from above. Result of a pure-culture inoculation using organism plated from a tumor on hop. Top of the plant cut away to show the tumor, the largest diameter of which was 5 inches. Necrosis not yet begun. Time, 3 months. The bacteria were introduced by a few needle pricks.


21
22

## PLATE VII

### Atrophy, Cachexia

23. Crown gall on sugar-beet. Pure-culture inoculation of 1907, using organism plated from a tumor on the Paris daisy. Tumors larger than the plant, which is very sickly. Time, $4\frac{1}{5}$ months.

24. Crown gall on sugar-beets. Pure-culture inoculations of 1913, using organism plated from a tumor on the hop (Humulus). Row of plants in the foreground (in front of screen) badly dwarfed and nearly dead (foliage yellow), and bearing tumors larger than themselves. Healthy control plants in the background showing above the screen. There was not a single dwarfed or diseased plant in the latter. Time, $3\frac{1}{2}$ months.


23
24

## PLATE VII*a*

### Atrophy, Cachexia

88. Crown gall on sugar-beet. Inoculations of 1916. Time, 2 months. Healthy controls in background. Hop organism. At the end of 3 months five of these plants were dead and two others were dying.


88

## PLATE VIII

### Degrees of Anaplasia. Notched and Cleft Nuclei. Giant Cells.

25. Crown gall on Paris daisy. Cross-section of a whorl of trachei in the center of a secondary tumor. It is surrounded by coarse-celled tissue and encloses fine-celled tissue which contains large deep-staining nuclei. Compare with figure 27, another whorl, but one showing large central cells.

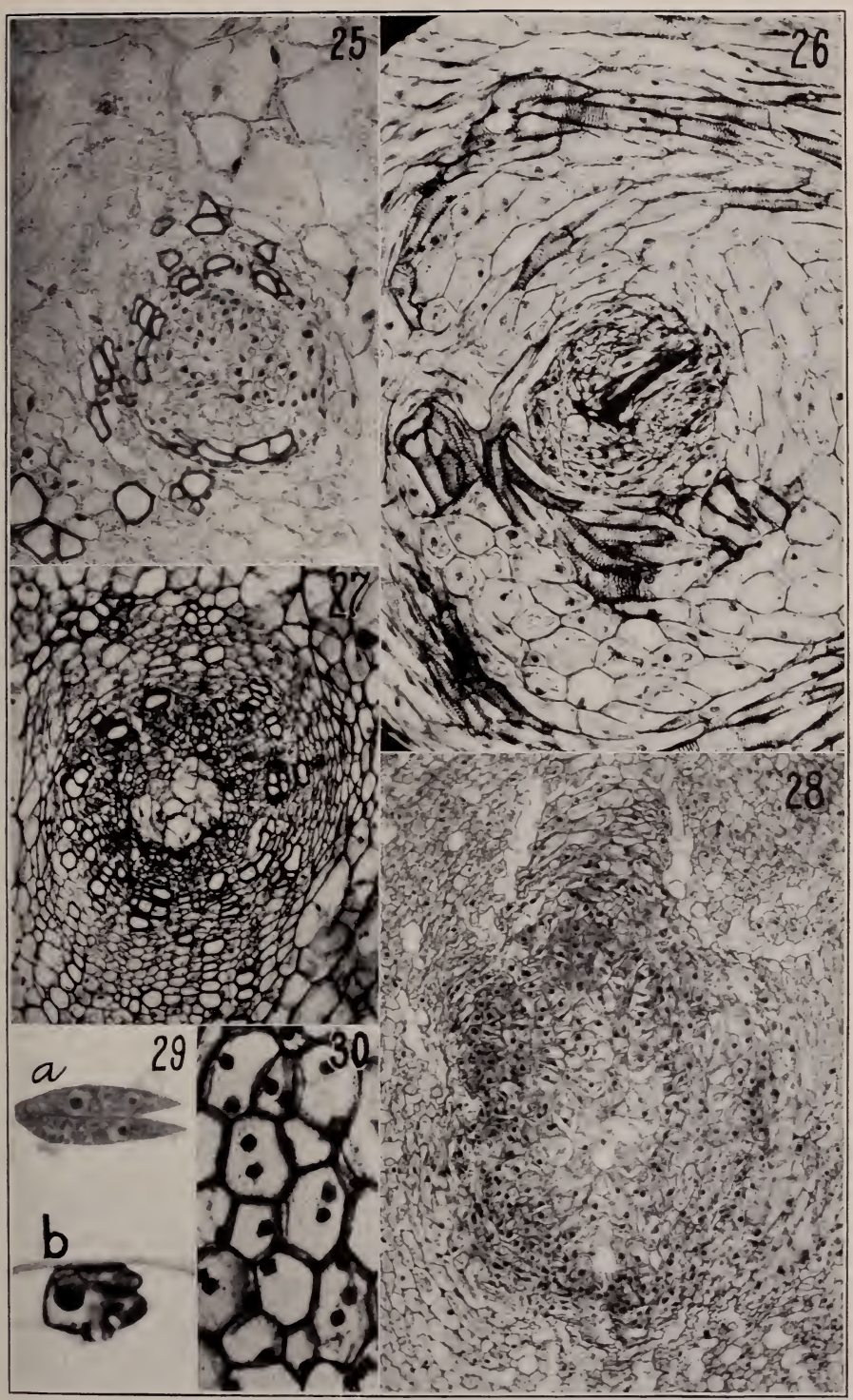
26. Crown gall on Paris daisy. Cross-section of inner part of a secondary tumor showing coarse and fine-celled tumor tissue and distorted trachei (polarity disturbed).

27. Crown gall on Paris daisy. Center of a secondary tumor in cross-section. Like figure 25 but showing a more perfect whorl of trachei and larger central cells. For orientation see Plate XII, figure 48.

28. Crown gall on Paris daisy. Cross-section of central tumor-strand in a secondary tumor, showing small rapidly dividing cells with large deep-staining nuclei.

29. Crown gall on Paris daisy. Nuclei showing various stages of amitotic division, i.e., notched and cleft nuclei. These also occur in rapidly growing normal tissues but are much less abundant. Material of lobed nucleus fixed in picroformol bichloride and stained in iron hematoxylin. The other fixed in Carnoy and stained by the Amyl-Gram method.

30. Crown gall of Paris daisy. Small portion of a tumor showing cells containing two and three nuclei.


25
26
27
28
29
a
b
30

## PLATE IX

### Plants Bearing Primary and Secondary Tumors

31. Crown gall on Paris daisy. Primary (inoculated) tumor at X. Secondary tumors in leaves *A* and *B*. In the stem, tumor-strands connecting *X* to *A* and *B*. Unruptured tumors on *B*.

32. Crown gall on Paris daisy. Plant inoculated at *X* where a primary tumor has developed. In the stem, tumor-strands passing from *X* into leaves *A*, *B*, and *C*, where secondary tumors have developed.

33. Crown gall on Paris daisy, photographed from above. Below at *X* (but out of focus) is the primary stem tumor, above are four leaves in each of which are several deep-seated secondary tumors (a total of 19) all of which are connected back to the primary tumor by deep-seated strands of tumor tissue.

34. Crown gall on Paris daisy. Plant inoculated at *X*. Secondary tumors in leaves *A* and *B*. Tumor-strand in stem. Cross-sections of such stems (as at F) when magnified appear as in figure 35 and the following illustrations.


31
B
4
3
2
A
1
X
X
32
C
A
B
X
33
X
34
S
B
S
4
3
F
2
Y
A
1
X
Y
X
Z

## PLATE X

### Parenchymatic Tumor-Strands

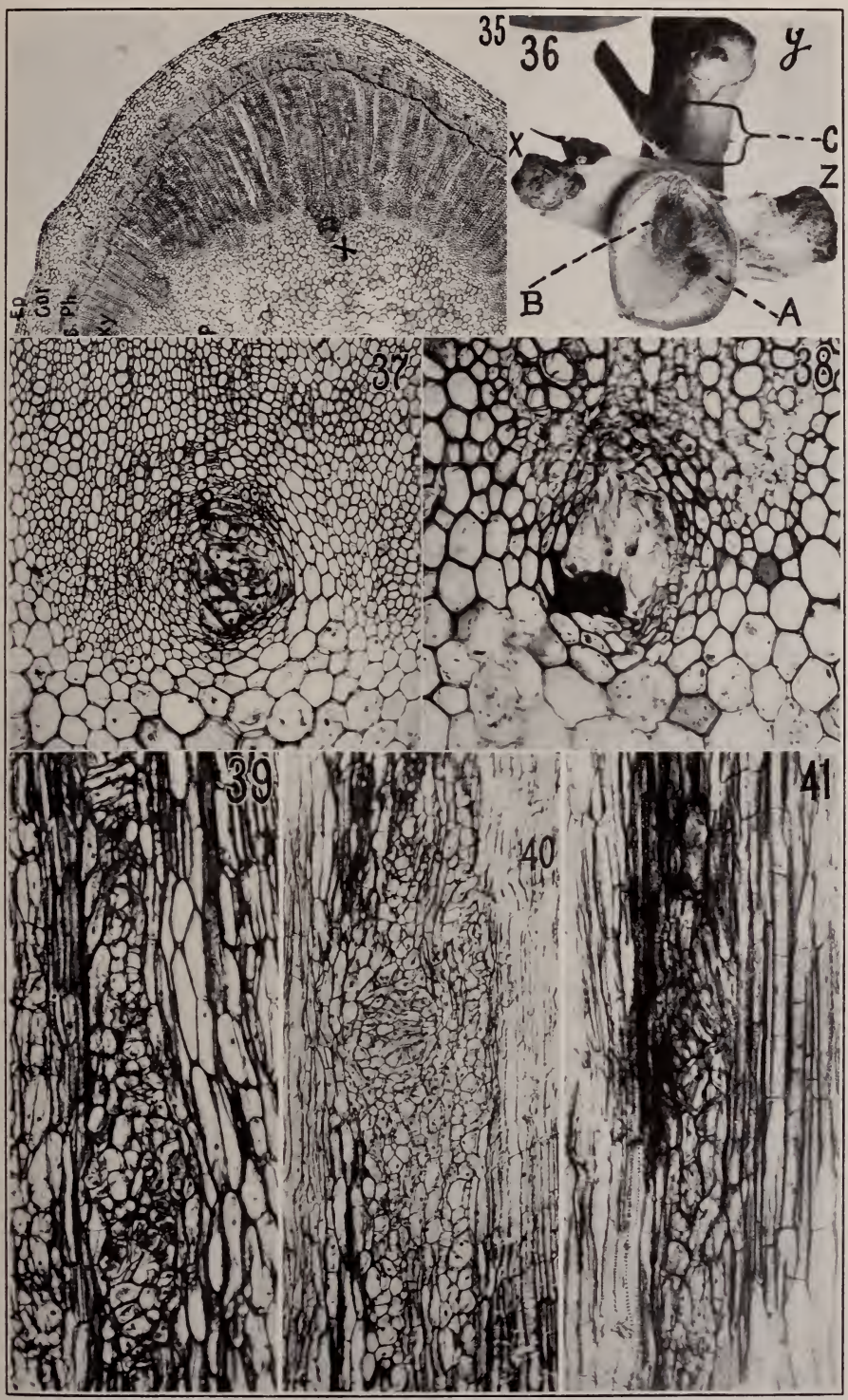
35. Cross-section of stem of an inoculated Paris daisy (general view), showing a tumor-strand at *X*. Stem otherwise normal at this level except for a slight thickening of the wood. Primary tumor on the stem below this point. Secondary tumors above in the leaves. *P*, pith; *Xy*, xylem (the wood cylinder); *C*, cambium (the chief embryonic proliferative cylinder, wood being produced from its inner face and bark from its outer face); *Ph*, phloem, the inner bark, called also bast or soft bast; *Cor*, cortex (the coarse-celled living outer bark of the young stem which is joined to the pith by many radiating plates of connective tissue known as medullary rays (the silver grain of wood); *Ep*, the one-layered epidermis (later in most woody plants this gives place to a many-layered, more resistant protective tissue known as cork).

36. Cross-section of stem of inoculated Paris daisy just above a primary tumor showing invading tumor-strands at *A* and *B*, in the vicinity of which the wood is thickened but not the bark. Strands unusually large, and dark in the photograph because they were green in the section. *X*, *Y*, *Z*, stubs of leaves out of which tumors have grown. These leaves bore deep-seated secondary tumors and were removed by clean cuts some weeks earlier for study after which these tumors developed. Strand *A* was traced into *Z*. One part of strand *B* was traced to *Y* and the other part to *X*. About natural size.

37. Magnified stem of inoculated Paris daisy between primary and secondary tumors showing appearance of a tumor-strand on cross-section. Pith below, inner wood above.

38. Like figure 37, but from another place in the strand, and more highly magnified. Tumor-strand in the center; above, infiltration; below, deep-staining trachei developed out of certain elements of the tumor-strand. The round bodies in the strand are large, deep-staining nuclei. The large-celled tissue at the bottom of the figure is pith. The vessels at the top of the figure are spirals of the inner wood, a different type of vessel from that occurring in the tumor-strand.

39–41. Crown gall on Paris daisy. Longitudinal section of three tumor-strands from as many inoculated plants.


35
36
y
C
X
Z
B
A
37
38
39
40
41

## PLATE XI

### Deep-Seated Young Tumors. Secondary Tumors Showing Structure of Primary Tumor

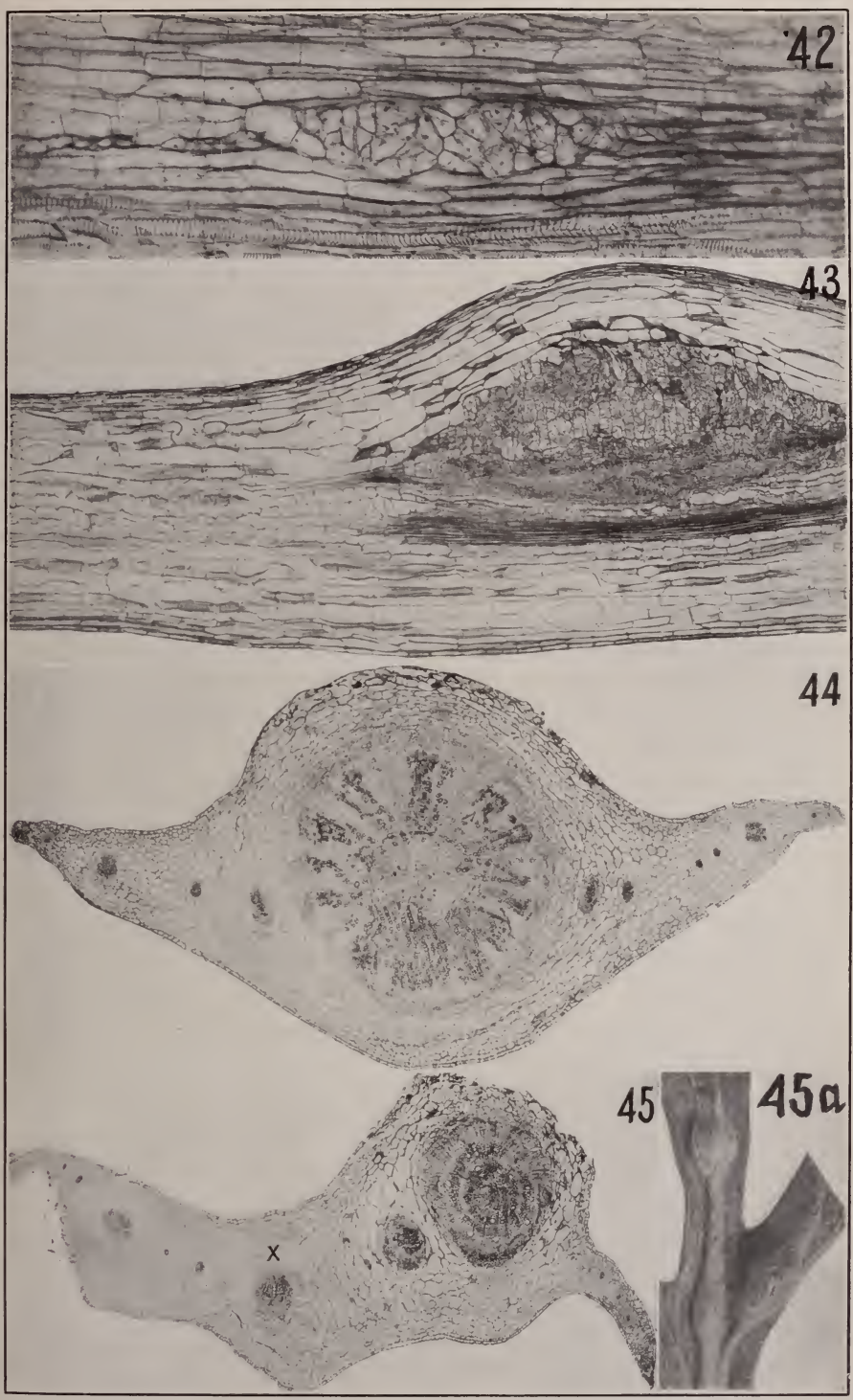
42. Longitudinal section of petiole of inoculated Paris daisy between primary and secondary tumors, showing tumor-strand and small tumor intruding between spiral vessels (at bottom) and trachei (at top).

43. Longitudinal section through petiole of Paris daisy showing early stage of a deep-seated secondary tumor. The normal tissues are crowded up but not yet ruptured.

44. Like figure 43, but in cross-section and secondary tumor further developed, but not yet ruptured to the surface. Tumor strand in the center (for appearance of such a strand when magnified consult fig. 5). The deep-stained (dark) parts are wood and show the scanty lignification of this pseudo stem. The infection occurred in the central leaf trace and the petiole is much swollen.

45. Like figure 44, but infection through side leaf traces. The unaffected (unilateral) central leaf trace may be seen at *X*.

45*a*. A slightly enlarged detail from fig. 33 showing several unruptured tumors.


42
43
44
45
45a
X

## PLATE XII

### Secondary (Leaf) Tumors Showing Structure of Primary Stem Tumor. Primary Tumor on a Leaf

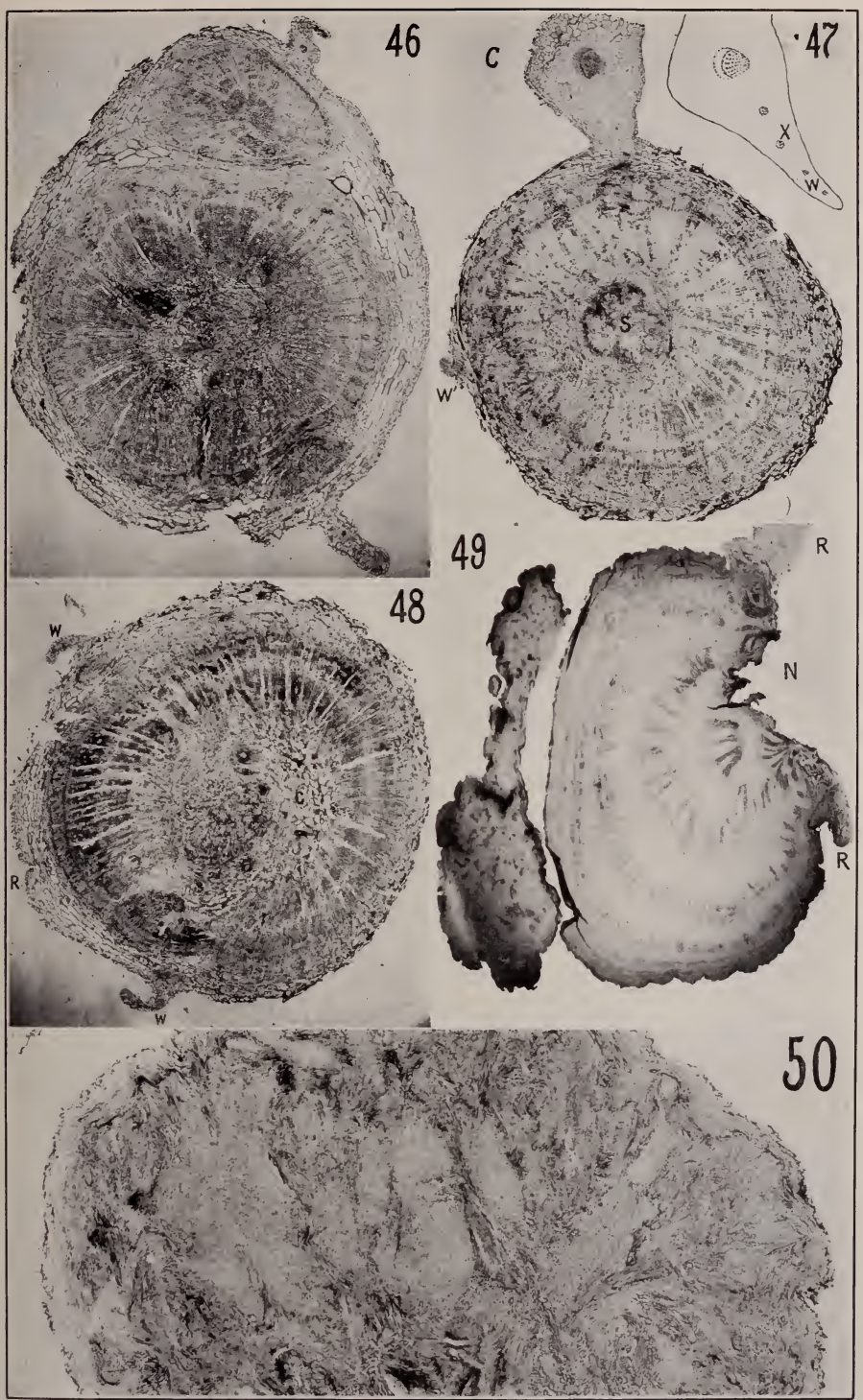
46. Inoculated crown gall of Paris daisy. Cross-section of an enormously swollen but not yet ruptured petiole containing 3 secondary tumors each with a central tumor-strand and a closed (stem) structure.

47. Inoculated crown gall on Paris daisy. A ruptured secondary leaf tumor in cross-section to show stem structure. *C*, center of petiole, where the tissues are normal. The size and shape of petiole (before invasion) is sketched in at right. *W* corresponds to *W'* on the tumor at the left (a remnant of normal tissue, i.e., one wing of the petiole). The tumor began in the leaf trace marked *X*. It is now naked but bears remnants of normal (dead) tissue here and there. The dark parts of the xylem are the only lignified ones. *S* is the tumor-strand.

48. Inoculated crown gall on Paris daisy. Cross-section of a secondary leaf tumor showing stem structure. At the right it has ruptured to the surface. To each side of *R* are remnants of the normal tissue, the normal wings of the petiole being at *W*. In the center is a tumor-strand in which are whorls of trachchei such as those shown in detail on Plate VIII. The coarse-celled tissues (*C*) at the right of this are cells of the cortex like those shown at *R* but surrounded and crushed.

49. Inoculated crown gall on sugar-beet. Cross-section of a secondary tumor that developed in a leaf stalk, the primary tumor being on the root. This shows root structure, i.e., 5 rings of vascular bundles. At the right, *R*, *R'*, are fragments of the normal leaf stalk, the central part of which (*N*) has decayed, this being the older portion of the neoplasm. The separated portion at the left is a part of the tumor.

50. Cross-section of an inoculated *primary* tumor on leaf of a Paris daisy. For comparison with figures 46 to 48. No stem structure. It is all a rapidly proliferating, highly blastomous atypical tissue. The multitude of minute specks, barely visible in the reproduction, are large, deep-staining nuclei. Tumor induced by a single needle prick. Section made crosswise of the longer axis of the leaf from which the tumor projected.


46
C
47
X
W
S
W
49
R
N
R
48
W
C
R
W
50

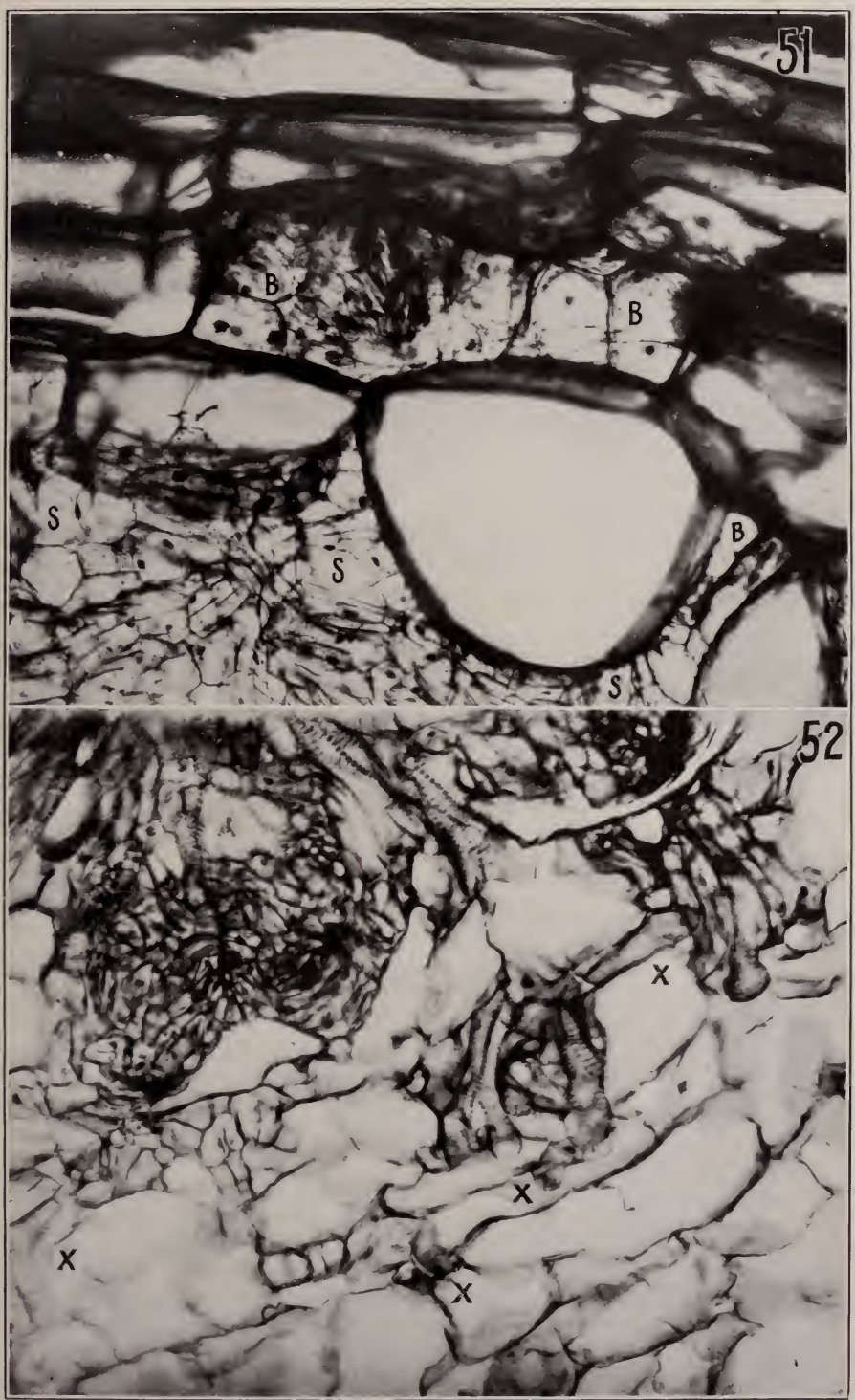
## PLATE XIII

### Further Examples of Infiltration

51. Margin of a secondary tumor (*S*, *S*, *S*) in leaf of Paris daisy showing the infiltration of small-celled tumor tissue (*B*, *B*, *B*) between the large cells of the outer part of the petiole. In the same section there is another small infiltration at the right of that here shown.

52. Margin of primary tumor (see fig. 6) in sunflower disk (back part), showing infiltration of tumor cells into the normal surrounding coarse-celled tissue. Trachei (stroma) abundant and pushing out along with the tumor cells. The youngest of these trachei (*X*, *X*, *X*, *X*) are very thin-walled and do not yet react to the lignin stain (methyl green).

(For another example of infiltration, see figure 59.)


51
B
B
S
S
B
S
52
X
X
X
X

## PLATE XIV

### Embryomata on Apple, Brassica and Pelargonium

53. Hairy root of apple from a nursery. Abnormally clustered and abnormally fleshy (soft) roots borne on small flat tumors out of which we cultivated an infectious organism indistinguishable from the crown-gall organism and with which both galls and hairy-root were produced.

54. Crown gall on stem of collards (Brassica sp.) produced by needle pricks introducing the organism plated from a tumor on Populus. The lower half of the lower tumor is thickly set with diminutive roots.

55. Crown gall on Pelargonium (red-flowered hothouse geranium). Inoculation into the apex of a shoot using the organism plated from a hop tumor. This portion of the resulting tumor is thickly set with a jumbled mass of imperfect leaf buds. The larger leaves at the top are also a part of the tumor. For a cross-section, see fig. 58.

55*a*. Teratoid tumors obtained by inoculating the leaf axils of Citrus sp. Below, one shoot is growing out of the tumor; at *X*, several shoots are pushing.


53
54
55
55 a
X

## PLATE XV

### Embryomata on Pelargonium

56. Crown-gall embryomata on geranium (Pelargonium). Plant from same series as figure 55 inoculated in the growing point, using organism plated from the hop tumor. Surface of the tumor white or brownish. It bears many clusters of tiny leafy shoots (*L*) the largest at *X* (with dead and dying leaves). Two weeks earlier this shoot was as vigorous as the large one in figure 55. It also shows clusters of abortive floral shoots (*F*). There were about 100 of these red shoots and serial sections made at *F'* show phenomena as astonishing as any ever seen in a malignant teratoid of the testicle, i.e., fused fragments of a variety of organs, oriented in various ways. The lower leaves (*D*, *D*) were dying. About one-half natural size. Time, 4 months.

57. Crown-gall embryomata on geranium (Pelargonium). Plant inoculated as in figures 55 and 56. Above, ordinary tumors; below, the needle entered the vicinity of dormant toti-potent cells (flower anlage) and rudimentary (red) floral organs have developed in various places, as at *X*. For sections of this tumor showing fragments of various organs, see figures 64 and 65.


57
X
56
F
F
L
L
L
F'
F'
L'
L'
X
D
D

## PLATE XVI

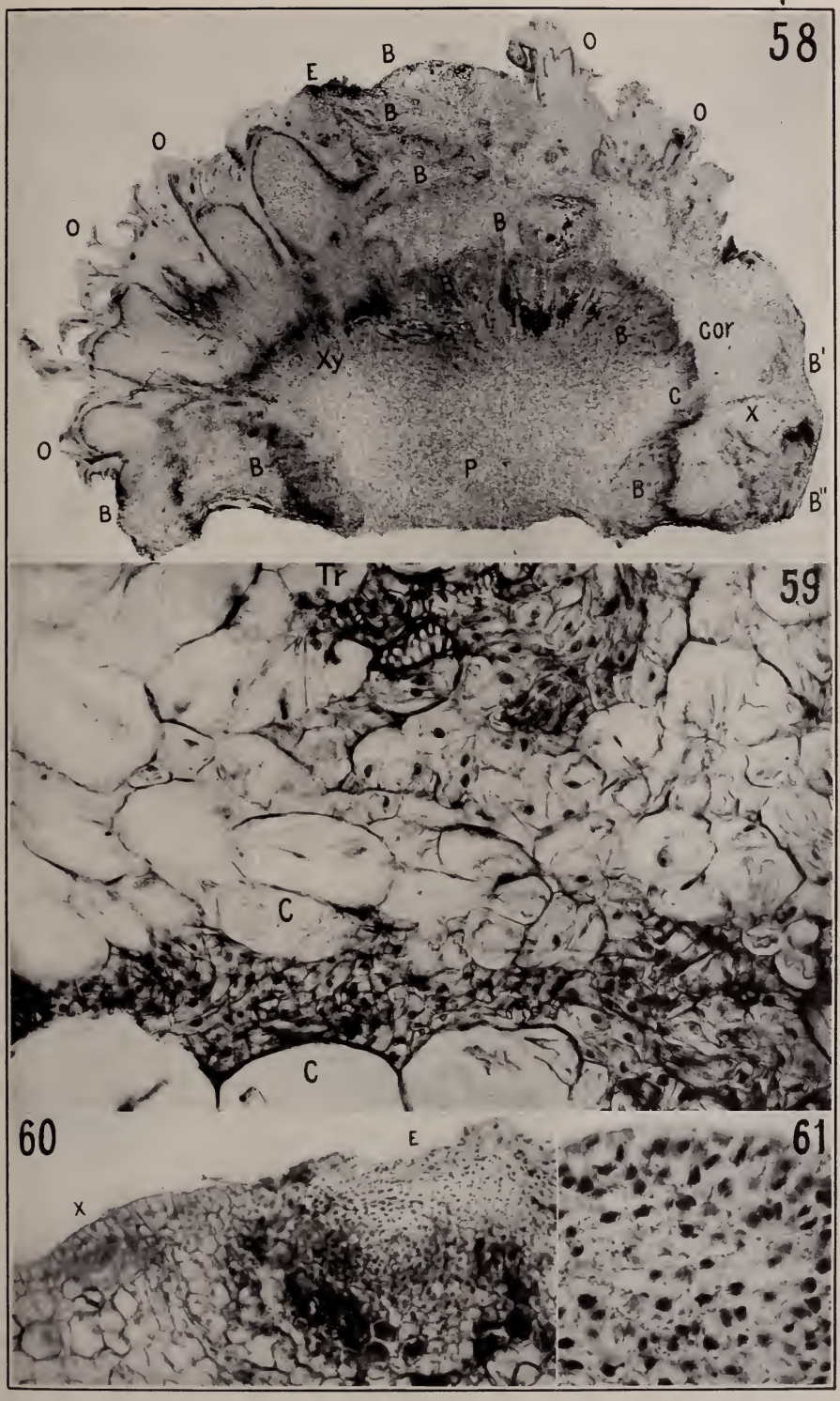
EMBRYOMATA ON PELARGONIUM. INVASIVE STRAND. EPITHELIOMATA (?)

58. Vertical section through the geranium tumor shown in figure 55. *P*. pith; *Xy*, xylem; *Cor*, cortex. Proliferating greenish organs at *O*, *O*, *O*, *O*, *O*, Blastomous portions *B*, *B*, *B'*, *B''*. These come to the surface below, at either side, and above in the center. Invasive strand at *X*. Curious surface tumors at *E* and apparent conversion of the epidermis into a neoplasm.

59. Detail from outer one-half of *X* in figure 58, showing the fine-celled, large-nucleate, deep-staining, rapidly multiplying invasive tissue wedged between large cells of the cortex (*C*, *C*). In the center above, blastomous tissue with distorted trachei (*Tr*).

60. A detail from figure 58 at *E*, showing a small peculiar surface tumor at *E*, and proliferating epidermis at *X*.

61. A detail more highly magnified from another one of the small surface tumors shown at *E* in figure 58. The distinction between epidermis and subepidermal tissues is lost.

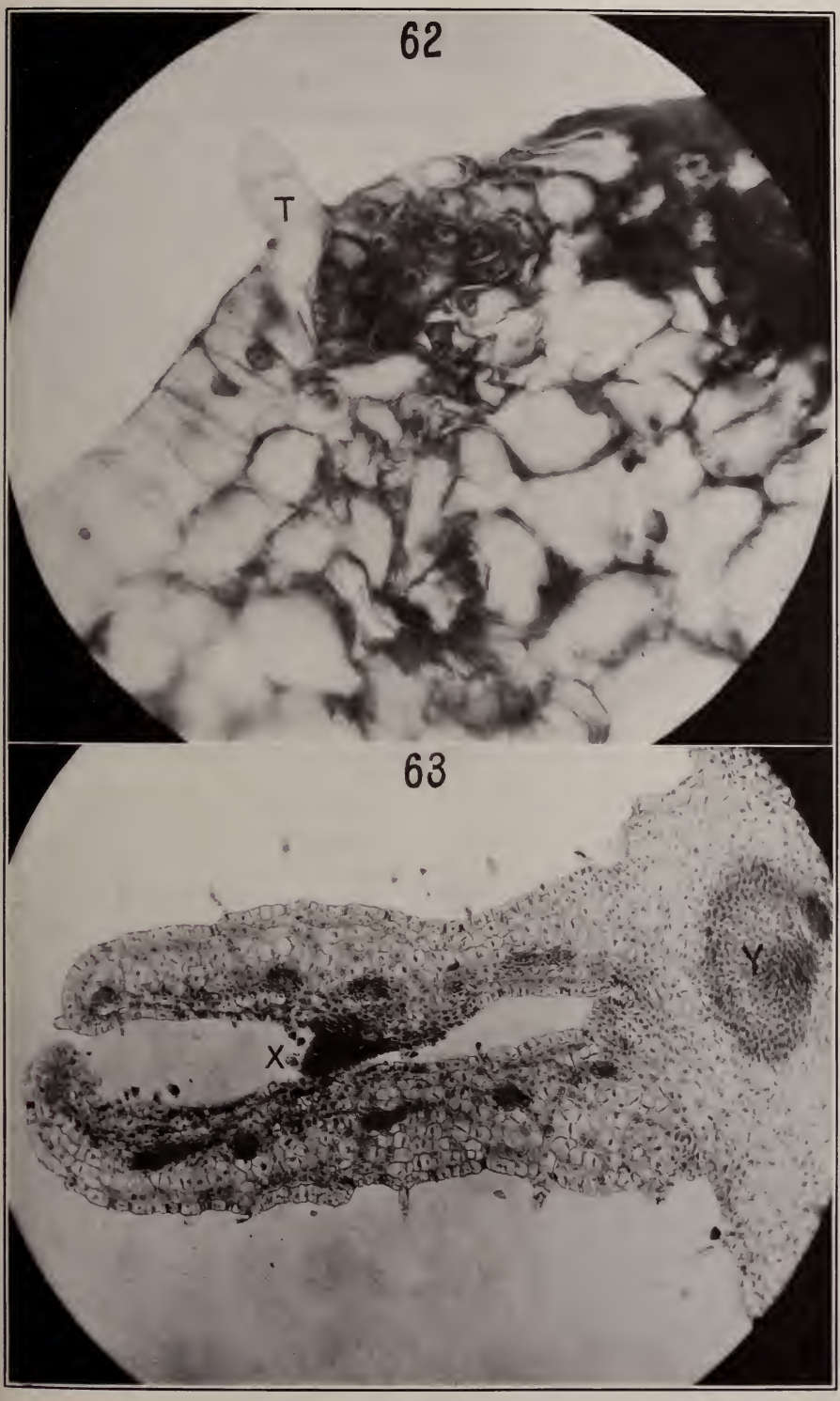

58
E
B
O
B
O
B
O
B
B
B
Xy
B
Cor
B'
C
X
O
B
P
B
B
B"
59
Tr
C
C
60
E
X
61

## PLATE XVII

### Embryomata on Pelargonium. Epitheliomata (?)

62. A detail from *X* in figure 60 showing the one-layered cylindric-celled normal epidermis on the left side of the trichome (*T*), becoming on the right side a several-layered, large-nucleate, deep-staining tissue dipping down into the subepidermal region. It will be observed also that these cells have become nearly isodiametric and have lost a portion of their polarity. They are now behaving like blastomous (epitheliomatous or carcinomatous) cells. Phenomena seen only in these sections and subject reserved for further study and experimentation, before a final decision is reached.

63. Surface view from section of lower (embryomatous) tumor shown in figure 57, illustrating floral anomalies. Organs covered by a normal looking epidermis. Numerous starch-bearing cells in subepidermal tissue. None of the tissues here shown are blastomous (that tissue lies a little deeper). At *X* and *Y* are deep-staining anlage of some sort, too rudimentary for determination; *Y* probably is ovarial in its nature, and *X* possibly is the anlage of an anther.

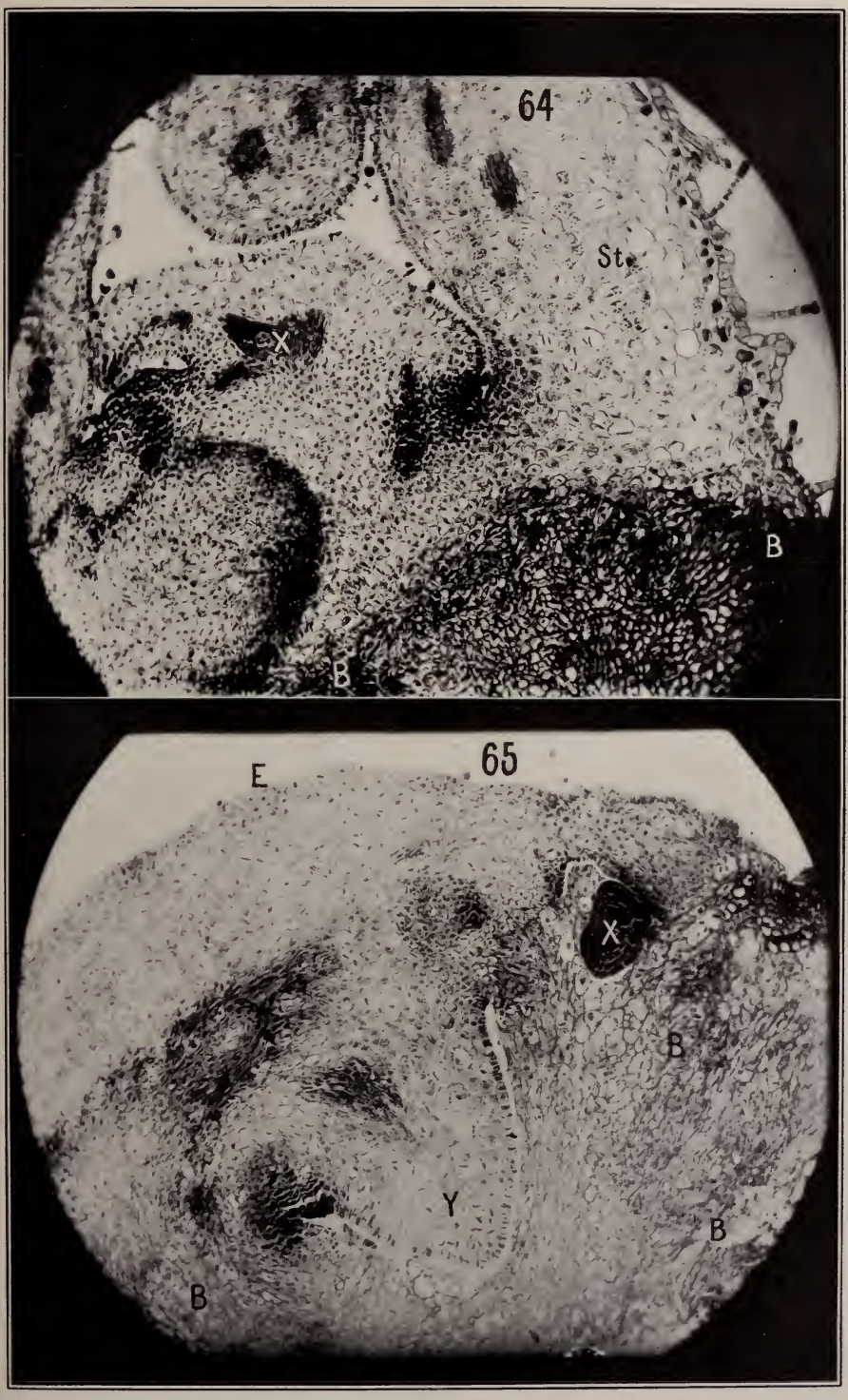

62
T
63
X
Y

## PLATE XVIII

### Embryomata of Pelargonium (Cont.)

64. Floral rudiments from surface of lower tumor shown in figure 57. Various deep-staining anlage visible, as at *X* which, I think, is the rudiment of an anther. Here the blastomous portion comes nearer to the surface than in figure 63. It is that deep stained atypical part of the tumor beginning on the lower right side of the section at *B* and extending to beyond the middle (*B'*). The rest of the section, while under the cancer stimulus, appears to be made up of rapidly dividing normal, or nearly normal, embryonic cells. Starch occurs in some of these cells as below and at the left of *St*.

65. From same series of sections as figure 64, introduced to show the disturbing influence at work in the tumor. Surface at *E*, covered by a normal looking young epidermis, at *X* a deep-staining rudimentary organ, probably the anlage of an ovule. At *Y* tissue twisted bottom up and lined by an epidermis. No parts here clearly blastomous except possibly at *B*, *B*, *B*. The jumbling in the depths of these tumors of embryonic fragments representing diverse organs of the plant is very striking.


64
St
X
B
B
65
E
X
B
Y
B
B

## PLATE XIX

### Embryomata in Tobacco

66. Tobacco plant 26 days after inoculation in the leaf axil at *X* with crown-gall organism, plated from tumor on hop, and passed through the sunflower. A tumor has developed where inoculated and this bears numerous leafy shoots. An uninoculated normal leaf axil at *A*. This inoculation was made about a foot below the growing point. No secondary tumors developed.

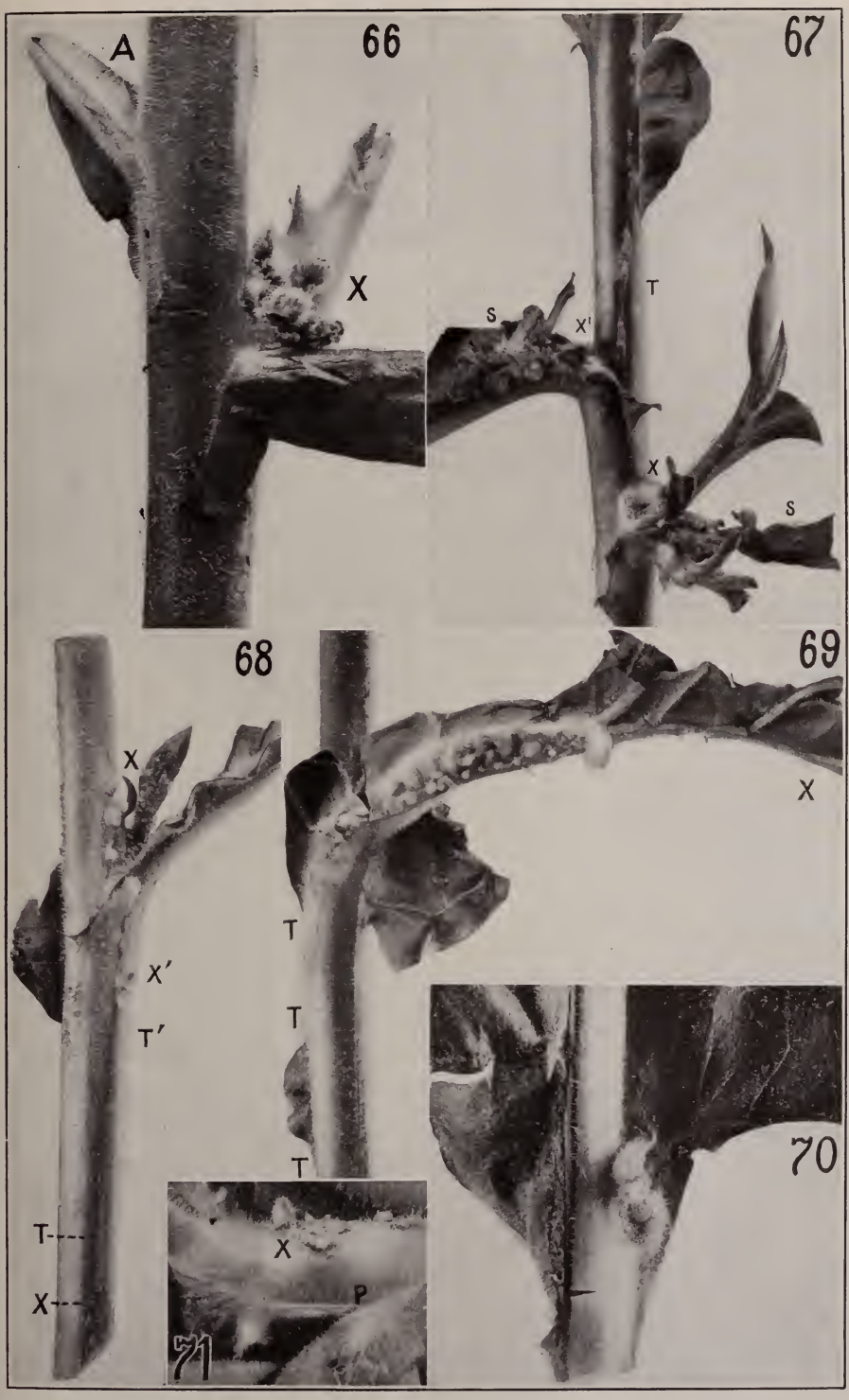
67. Tobacco plant from same series as figure 66, and also 26 days after inoculation which was at the top of the plant in the axil of young leaves. Both axils developed small, shoot-bearing tumors (*X*, *X'*), and sent tumor-strands into their subtending leaves (*S*, *S*), from which leafy tumors have ruptured to the surface. Also from *X'* a tumor-strand (*T*) passes upward in the cortex of the stem and this has sent to the surface in this part of the stem 16 small tumors some of which bear leaves. The infection extended upward through 5 internodes as shown further in figures 68, 69, and 70, ending in the midrib of a leaf. The tumor-strand in this plant was near enough to the surface to show through as a narrow translucent band of tissue, and it has the structure of a stem in miniature. In its upward progress it caused the development of more than 30 tumors, many of which were teratoids.

68. Part of stem next above that shown in figure 67. The tumor-strand in the cortex continues sending out occasional tumors as at *X*, *X'*, *X*, and some of these bear shoots (as at *X'*) while others do not. The track of the tumor-strand, as at *T*, *T'*, is clearly visible through the translucent epidermal and subepidermal layers.

69. Upper portion of infected tobacco stem shown in figure 67, i.e., part next above figure 68. The tumor-strand continues near the surface on the left side (*T T T*) passing into the midrib of the leaf from the lower surface of which organ has burst out a long irregular tumor, bearing many diminutive leafy shoots. The last visible tumor from this strand is at *X* and this also bore a green shoot.

70. Upper surface of one of the tobacco leaves (same plant as figs. 67–69), showing a deep-seated small tumor that has ruptured to the surface and bears 5 small greenish shoots, and two nodules destitute of leaves. For a longitudinal section see figure 74.

71. Tobacco stem inoculated by deep punctures below a leaf, 7 needle punctures yielded the ordinary tumors; the eighth puncture (one nearest the leaf) yielded a shoot (*X*) bearing 6 leaves. The wing of the leaf extends downward to the point marked *P* but the middle vascular portion passes out of the phloem-xylem region above the point marked *X* where the shoot originates.

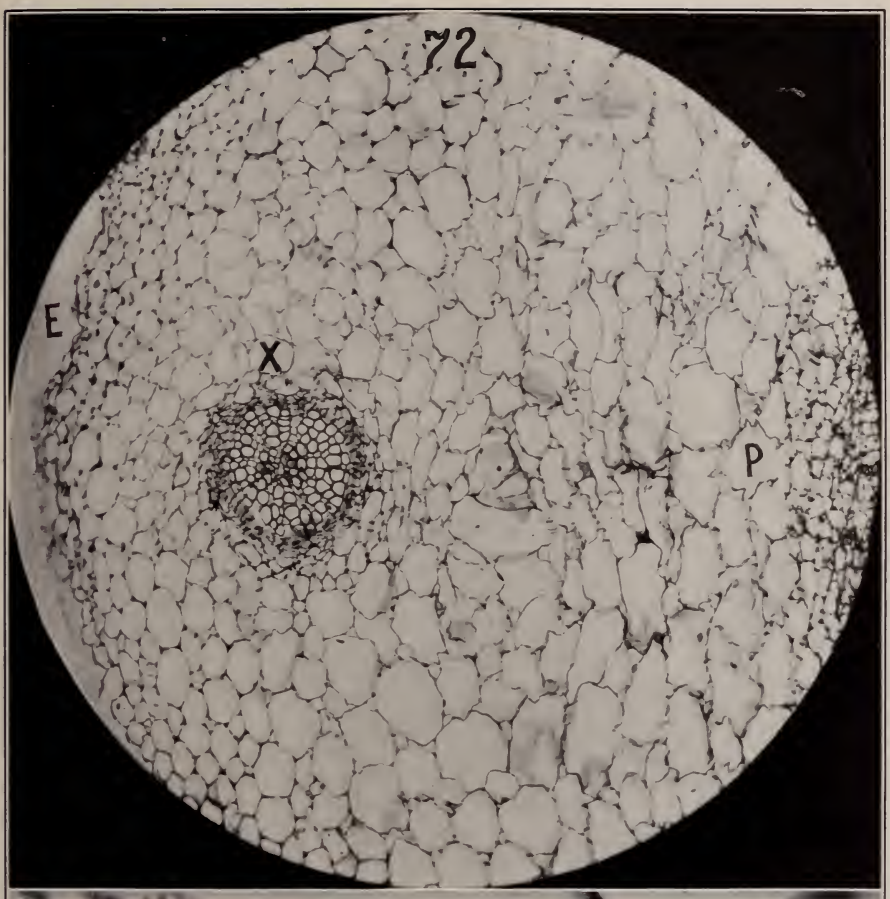

A
66
X
67
S
X'
T
X
S
68
X
X'
T'
T
T
T
T
X
69
X
70
T
X
71
X
P

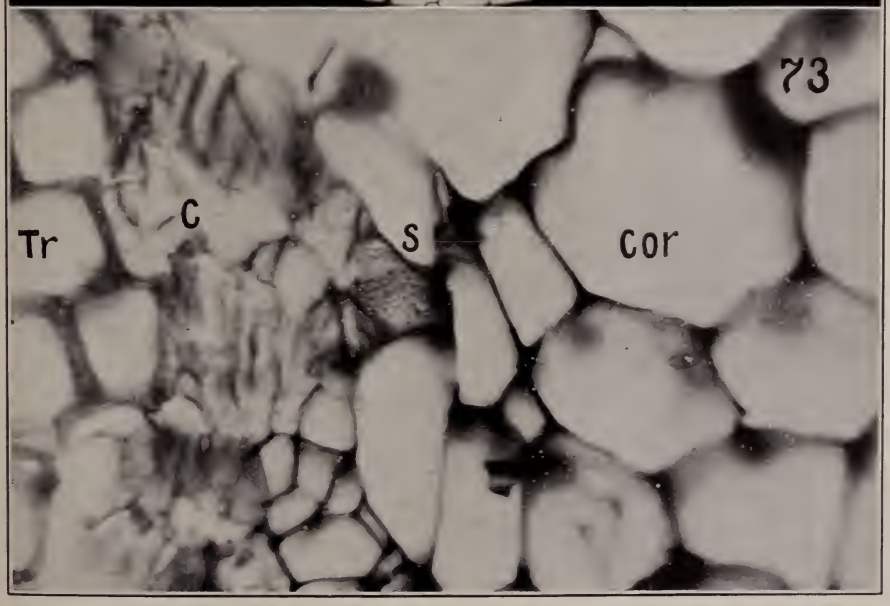
## PLATE XX

### Embryoma on Tobacco—Stele-Like Tumor-Strand

72. Cross-section of cortical part of tobacco stem shown in figure 68 at *T'*. Epidermis at *E*. Extreme outer part of phloem at *P*. At *X* is a cross-section of the tumor-strand showing perfect stem structure.

73. A detail from the outer part of the tumor-strand shown in figure 72. *Tr*, trachei; *C*, cambium; *S*, a sieve plate; *Cor*, normal cortex surrounding the tumor-strand.


72
E
X
P

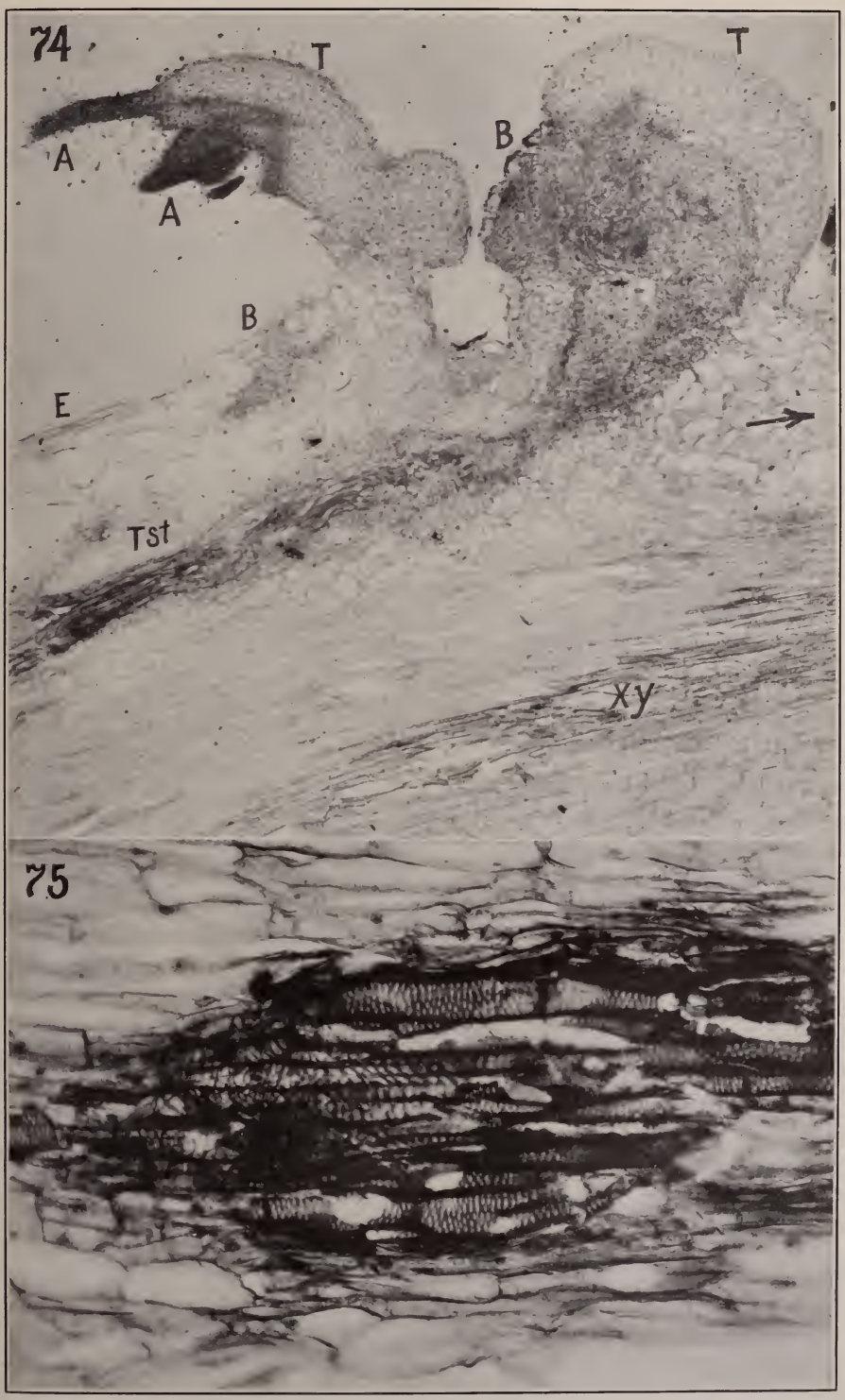

73
Tr
C
S
Cor

## PLATE XXI

### Embryoma on Tobacco—Stele-Like Tumor-Strand

74. Radial longitudinal section through midrib of tobacco leaf shown in figure 70, the knife passing through the tumor-strand, and through some of the outgrowths. *E*, epidermis; *T st*, tumor-strand; *T*, *T*, teratomatous outgrowths; *A*, *A*, deep-staining anlage; *B*, *B*, blastomous parts; *Xy*, normal xylem. At another level the tumor-strand continues in the direction of the arrow.

75. Detail from tumor-strand shown in figure 74. Beginning in the middle, at the top, the order of tissues is as follows: Phloem, cambium, trachei, spirals, trachei, cambium, phloem. At the bottom, normal cortex of the stem.


74
T
T
A
A
B
B
E
Tst
Xy
75

## PLATE XXII

### Embryomas on Tobacco

76. Embryonic teratomata in tobacco. Same series as figure 67 but at end of 40 days. Inoculations by needle-pricks in the upper leaf axils. Special attention is called to *X*. This leaf bears a leafy tumor (*B*) in its axil, a smaller one at *T*, and from the back of the midrib, a well developed shoot (*S*), which grows directly out of the leaf without visible connection with a tumor. All of these shoots are abnormally swollen at the base and perishable.

76*a*. Atypical teratoid (shoot-bearing, leafy) tumor produced entirely out of leaf tissues by inoculating *Bact. tumefaciens* into the middle of the midrib of a tobacco leaf. Twenty-seven of these were produced on the leaves of one plant, all by local (leaf) inoculations, by needle-pricks.


76
B
T
X
S
76a

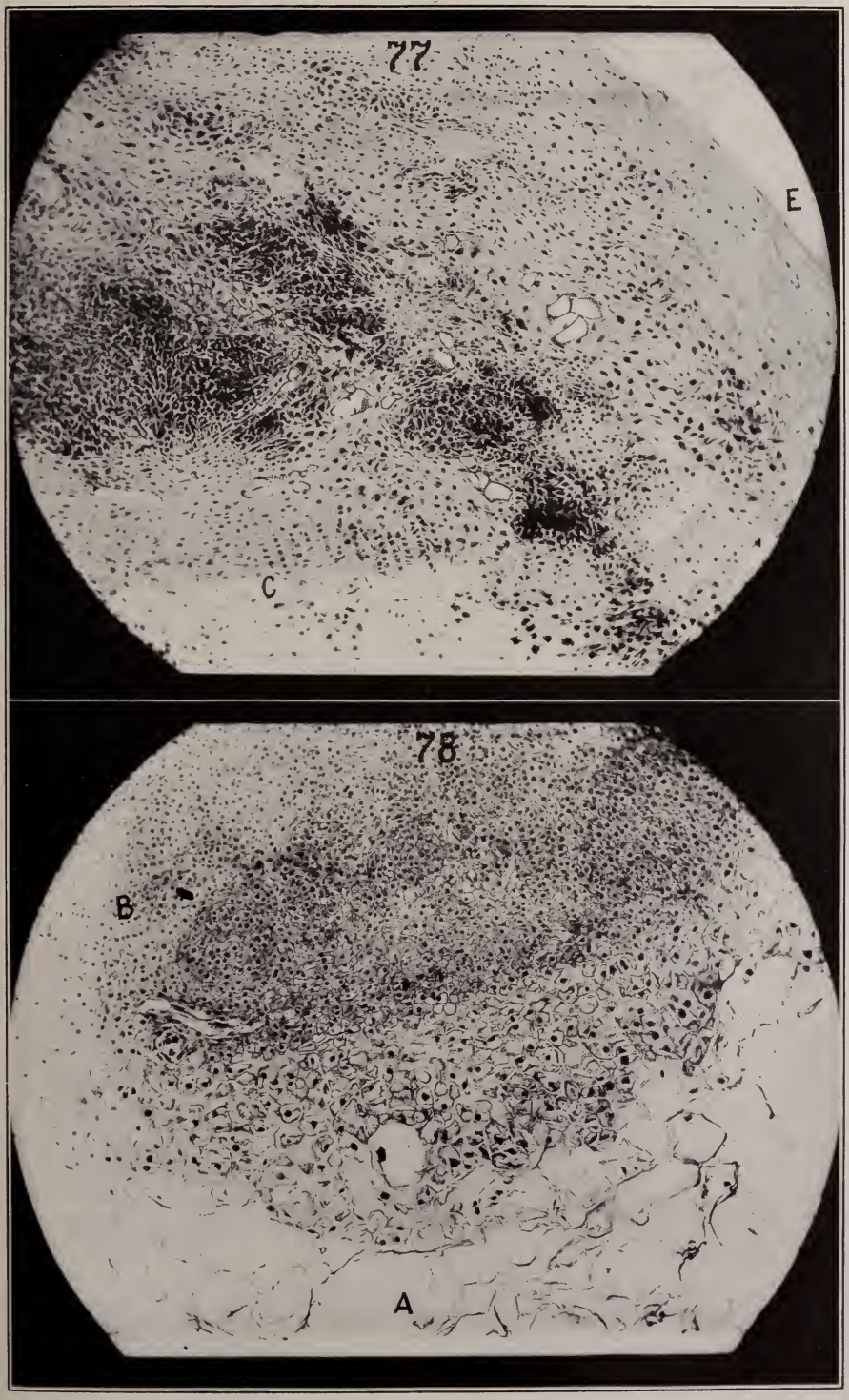
## PLATE XXIII

### Mixed (?) Tumor in Tobacco

(Details at *S* and *E* from the superficial tobacco tumor shown in figure 3, introduced as proof that the tumor contains different types of blastomous cells, an outer growth suggesting epithelioma and a deeper growth suggesting sarcoma. Same magnification.)

77. Superficial part. Normal epidermis at *E*, the remainder blastomous except a few cells immediately under *E*. Trachei in cross-section are scattered about, and above *C* is tissue that looks like cambium and was possibly derived from cork cambium. The deep-staining cells are more compact and less rounded than those in figure 78 and contain more protoplasm outside the nucleus.

78. Inner part. Roundish cells with large deep-staining nuclei lying in a clear cytoplasm. Lower part of figure (*A*) shows unchanged cortex; the upper part (*B*), small blastomous cells in very active growth. Between are larger blastomous cells, i.e., a transition tissue, *A* being converted into *B*.


77
E
C
78
B
A

## PLATE XXIV

### Animal Experiments

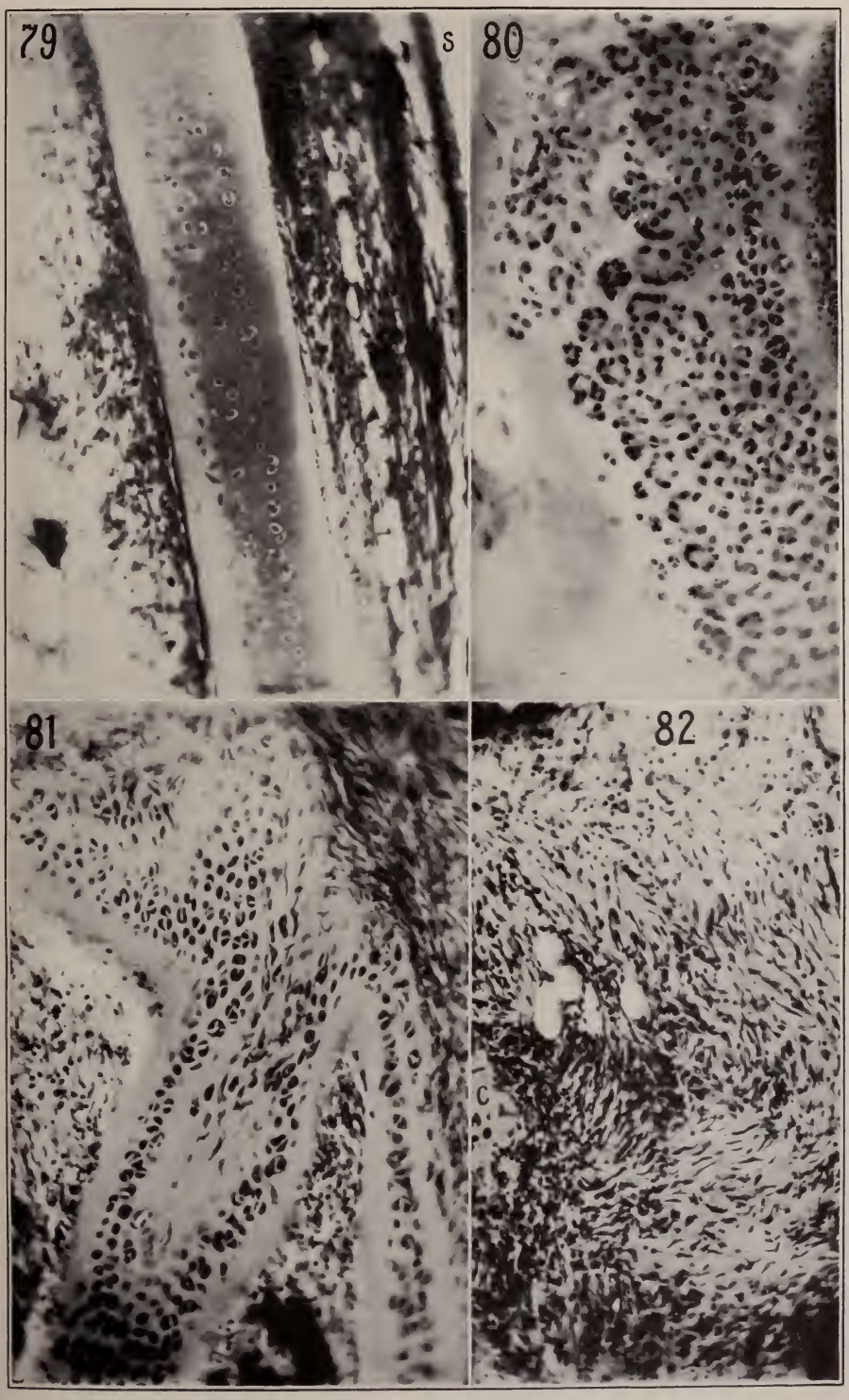
(Trout inoculation No. 228, Slide 920–17. Poplar organism, inoculated 22 days.)

79. Normal sclerotic of the eye composed of cartilage and staining blue with haematoxylin eosin. Surface of the eye at *S*.

80. Another part of the same inoculated eye, cells of the sclerotic proliferating (chondromatous). Many nuclei in some of the cells. Tissue stains red with haematoxylin eosin. Inflammatory condition on the right.

81. Same eye as figures 79 and 80, but a portion showing further reversionary changes in the sclerotic, i.e., appearance in it of fibrous cells.

82. Same as figures 80 and 81, but here the entire sclerotic has been converted into rapidly proliferating fibrous tissue, except a small part (*C*) at the left. This is especially interesting because in trout the embryonic structure of the sclerotic is not cartilage but fibrous tissue.


79
S
80
81
82
C

## PLATE XXV

### Animal Experiments

(Trout Inoculations Nos. 228 and 221)

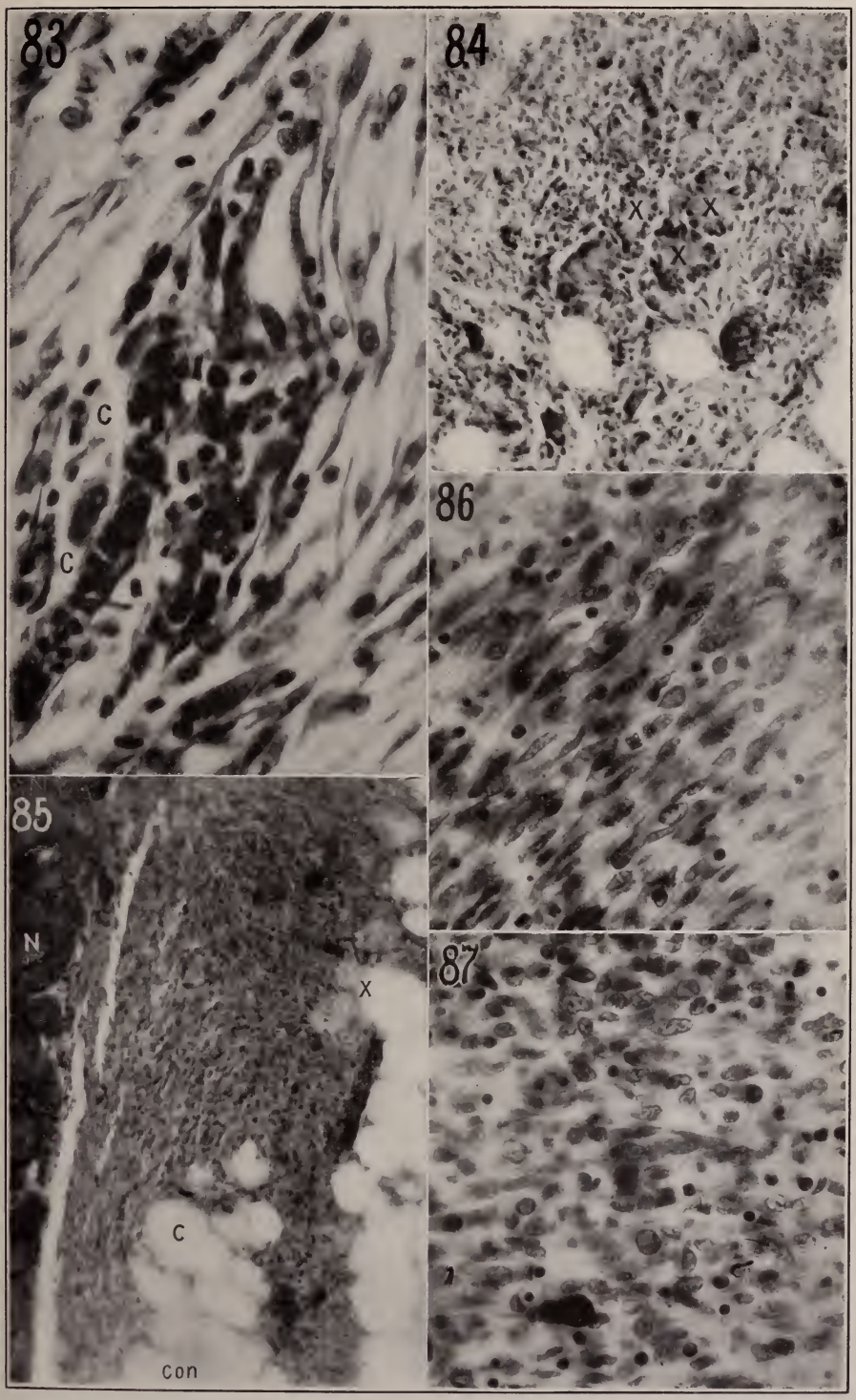
83. Fibrous tissue developed from sclerotic of trout with a capillary forming in it on the left at *C*. Same as figure 82 but more highly magnified. A cross-section.

84. Muscle from same eye as figures 79 to 83. The muscle fibers have been destroyed and absorbed, but the outlines of some of them remain as at *X*, *X*, *X*, between which the cells of the fiber sheaths have proliferated. A cross-section.

85. Same as figure 84, showing infiltration of connective tissue between muscles, i.e., between the normal muscle (*N*) at the left and the disorganized one shown in figure 84 which lies in the direction of *X*. Capillary below *X*. A cross-section.

86. Margin of a mass of new tissue in the inoculated eye of trout No. 221 at end of 10 days. This mass which stains blue with haematoxylin eosin, developed principally in the region of the choroid which was torn into pieces by its growth. It is on one side of the eye and measures approximately 5 mm. by 4 mm. by 2 mm., thinning out in places. It contains very few blood corpuscles, nor is there much vascularization. A cross-section. × 500.

87. Another view from the same compact mass of new tissue as that shown in figure 86. Some dark granules are scattered about in it. Giant cells occur and many lobed or notched nuclei. × 500. This eye protruded about a centimeter but was not ruptured.


83
C
C
84
X
X
X
86
85
N
X
C
con
87

# TERATOMATA OF THE BRAIN[1]

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Clinically, the histopathology of brain tumors is of very little importance, since the symptoms and the prognosis in any given case depend entirely on the location and the size of the neoplasm. But scientifically, all types demand the closest study. Of all the variety of tumors encountered in the brain, the teratomata are unquestionably of the greatest interest and, at the same time, of the rarest occurrence.

Mallory (1) defines a teratoma as a tumor that develops slowly from cells which are inherently capable of producing an entire embryo and which undergo a large amount of differentiation. Aschoff (2) defines this type of growth as a tumor which contains derivatives of the three primary germ layers. He includes not only the cystic teratomata (dermoid cysts) and the solid teratomata, but mentions also the fact that many authorities include in this group such tumors as the cystic adenomata of the ovary. These tumors, he states, arise from cells which potentially are similar to the fertilized ovum. Askanazy (3) regards a teratoma as a tumor composed of multiple tissues or organs explicable only by an early embryologic derangement. He concludes from his very careful studies of teratomata in ovaries, testicles, fallopian tubes, peritoneal and pelvic cavities, sacrum, mediastinum, neck, orbit, cranium, and extremities, that they arise from aberrant multipotential, or rather totipotential, cells

[1] Read before the section of Pathologists and Bacteriologists at the Sixteenth Annual Meeting, at the Congress of American Physicians and Surgeons, Washington, D. C., May, 1916.

which were separated from and included in the developing ovum before the gastrula stage. Cells becoming aberrant after the gastrula stage are capable of developing only into the so-called "mixed tumors." From the locations of the teratomata above mentioned, given in the order of their frequency, it will be seen that the intracranial ones are almost the rarest of the whole series; even the most frequent of the series, however, are rare.

One theory which has been expounded to account for the origin of the teratomata is that which would refer them to the fertilization of the polar bodies. This is not tenable because there are cases on record in which three, four, and even five teratomata have been found in a single individual. Such an occurrence at once involves the great difficulty of explaining the origin of so many polar bodies. In spite of the marked variation in structure, all teratomata may be characterized by their composition of elements derived from the three primary layers. One of these elements may possess a predominating energy for proliferation and thus tend to obscure the others.

Two types of teratomata are described (1): 1, Teratoma adultum, in which the tissues present may correspond in the stage of development to that of the host, and 2, Teratoma embryonale, in which the tissues are of an early embryonic structure. In the latter group there is present a greater variety and a more diversified arrangement of the tissues because there is more active proliferation and less secondary atrophy. The former variety is more frequent in the ovary, for example, while the latter is more commonly found in the cranial cavity.

The earliest case of teratoma of the brain which I found reported in the literature is that of R. Maier (4). His case is that of a ten-weeks-old boy in whom there was present a nodular, cystic tumor the size of an apple inside the left cerebral hemisphere. It appeared to arise from the depths of the brain tissue itself, involving the floor and wall of the lateral ventricle. Histologically, the tumor consisted of epithelium with sebaceous glands and fine hairs, connective tissue showing sarcomatous changes, cartilage, and bone. The tumor was nodular and contained cysts.

Weigert (5) describes a teratoma of the brain involving the epiphysis in a 14-year-old boy. The tumor was nodular, about the size of an apple, and was partly solid and partly cystic. Histologically, it was composed of epidermis, sweat and sebaceous glands, cartilage, fat, nerve bundles, bundles of smooth muscle fibers, connective tissue, and cysts lined with cylindrical epithelium. The tumor was rather deep-seated, lying in front of and below the corpora quadrigemina.

Falkson (6) describes a tumor about the size of an apple, growing out from the floor of the third ventricle in a boy 16 years old. The tumor was nodular and gave the appearance of a multilocular cyst. It contained cysts and cartilage in a stroma composed of spindle cell sarcoma. The pineal gland could not be located, and the author could not determine whether the tumor originated from this gland or from the choroid plexus. It is questionable if this can properly be considered as a teratoma unless some other elements were overlooked.

Beck (7) found a teratoma the size of a walnut in the hypophyseal region of a woman 74 years old. This tumor contained 14 teeth.

Strassmann and Streckner (8) discuss an accidental finding of a teratoma the size of a walnut in the right ventricle of the brain at an autopsy on a three-year-old boy. This tumor is interesting because of the variety of tissues present. Histologically, it showed cysts lined with one or more layers of epithelium, acinous and tubular glands, nerve bundles with multipolar ganglion cells, glia cells, striated and nonstriated muscle, connective tissue, fat, cartilage, bone, blood-vessels, and lymphatic tissue.

Gauderer (9), in a monograph, cites six cases of tumors of the epiphysis of which only one (Weigert [5]) is a true teratoma. His own case is that of a 12-year-old boy. The growth, which measured 3.5 x 2.5 x 2.5 cm., involved the epiphysis and compressed both optic thalami and the corpora quadrigemina. He could find no normal epiphyseal tissue, but he assumed that some of the gland-like structures with small lumina (some of which contained concretions) were atypical remnants of the pineal gland. Of special interest was the presence of what he considered a

metastatic nodule in the optic chiasm. He does not, however, describe the histologic findings in this nodule. In the main tumor, he found connective tissue cells, some with abundant cytoplasm, and small areas of round cells suggesting leucocytes. Some cysts were lined with cylindrical cells, others with squamous cells showing cornification. I think it quite probable that the bodies resembling concretions were nothing more than well hyalinized epithelial pearls. There was present, also, hyaline cartilage, bone surrounded by spindle and round cells, fat cells, here and there nonstriated muscle fibers, and some nests of epithelium which strongly suggested hair follicles.

Saxer (10) found a tumor 8 x 10 x 12 cm. in a seven-weeks-old baby girl who had had a normal delivery. The head at time of birth was normal. The tumor involved the third ventricle and was attached to the tela choroidea; it was partly solid and partly cystic. The pineal and hypophysis were normal, the cerebellum slightly compressed. Microscopically, the tumor showed a large mass of fetal brain tissue in different stages of development while scattered throughout it were cyst-like structures lined with epithelium, varying so greatly in their structure that they almost defied description. Some cysts were lined with columnar epithelium which contained goblet cells; others appeared like the glands of the skin. The connective tissue, also, gave variable pictures, part of it looking like Wharton's jelly. In some areas, red blood cells appeared to be originating in the connective tissue. Striated and nonstriated muscle, cartilage, bone, and giant cells were present. In discussing the development of this tumor, he suggests that it probably arose from a fetal implantation or from an abnormal inclusion of portions of the outer and middle germ layers during the closure of the medullary plate, and that later this became covered by the cerebral hemispheres. It seems to me that some of the gland-like structures, especially those which contained cells suggesting goblet cells, were probably of entodermal origin, and that the tumor actually contained elements of all three embryonic layers.

Eberth (11) found an unsuspected tumor containing fat, muscle, nerve bundles, and lymphoid tissue, during an autopsy on

a woman 75 years old. This was situated on the inner surface of the dura mater, immediately over the right cerebral hemisphere. Such a growth might easily be overlooked, as it was only about the size of a split pea. This tumor is of interest because it shows that misplaced cells may remain in the body without rapid growth. He considers the tumor a teratoma which arose from the mesoderm, a somewhat paradoxical conclusion, because he limits the origin to cells from a single germ layer. Furthermore, the nerve bundles could scarcely have originated from the middle germ layer.

Askanazy (12) discovered a hemorrhagic tumor the size of a walnut in a young man 19 years old. It was attached in the region of the pineal gland; the pineal itself was absent. There was softening of the left pulvinar and left geniculate body. Microscopically, typical chorionepithelium-like cells of both the syncytial and Langhans types were found. He did not examine the testicles for tumor, but felt convinced, nevertheless, that the tumor was primary in the pineal. He explains the development of chorionepitheliomata by stating that chorionepithelium, like neuroepithelium, is a specific fetal structure, and that consequently very early cell inclusions may have the potentiality of reproducing these types of cells. Atypical pineal tissue was present on the periphery of the tumor. The blood vessels were very abundant and in close association with the tumor cells, a condition very characteristic of chorionepitheliomata.

Hecht (13) reports a case of tumor of the hypophysis in a girl 11 years old. He mentions the fact that Boyce and Beadles, in a large collection of cases of tumor of the hypophysis, include only three teratomata. One of these (Beck [6]) I have already cited. His own case presented a tumor about 2 x 2.5 cm. in size, fused with the optic commissure, corpora mammillaria, and floor of third ventricle. He states that "the teratomata are in their nature a fetal type of tumor" and "very probably derived from an embryonal inclusion." The tumor, which was irregular, heterogeneous, and contained small calcareous particles, showed upon microscopic examination a loose stroma composed of stellate cells and containing gland-like structures, osteoid

tissue, and true bone. Some of these gland-like structures were lined with columnar epithelium, others contained questionable epithelial pearls. Some portions of the bone were highly differentiated, showing Haversian canals and cellular marrow spaces.

Cushing (14) cites a case of an interpeduncular teratoma of the pituitary in a girl 16 years old. The tumor was the size of a golf ball, and composed of cartilage, bone, and myxomatous tissue. Without the presence of any other elements, this growth could scarcely be classified under the teratomata, since it contained only structures of mesodermic origin. His second case (15) is subject to the same criticism. A large friable tumor, originating in the hypophysis, was present in a man 35 years old. The symptoms were of four years' duration. Histologically, the tumor showed myxomatous connective tissue, embryonic cartilage, and a few bone cells.

Teutschlaender (16) describes a tumor composed of cornifying stratified epithelium, sebaceous glands, hair, and a large accumulation of sebaceous material. It measured 9.2 x 8.2 x 7.7 cm. and was situated at the base of the brain in the left frontal region between the dura mater and the pia. He states that dermoid cysts, the type to which his tumor belongs, occur in the brain principally at the base. His theory is that such tumors arise from an inclusion of superficial epithelium during the development of the labyrinth. He employed a rather ingenious though not very convincing way of proving the relation of these types of tumors with the development of the ear; a very careful chemical analysis of the tumor material was made, which showed that the character of the fatty material corresponded very closely to the cerumen of the ear.

Burmeister (17) found a tumor 4.7 x 2.5 x 3.5 cm. in a man 60 years old, located in the right ventricle and attached to the choroid plexus. It was composed principally of hyaline cartilage and was covered by a multilayered epithelium. He thinks that the mesoblastic cartilaginous elements were carried in during the embryonic "inthrust" of the velum interpositum. This, therefore, is not a true teratoma.

The case to be reported in this paper is that of a young colored man, A. J., 19 years old, who was admitted on the service of Dr. E. M. Hammes at the City and County Hospital, St. Paul, on May 22, 1915. The history was to the effect that on the evening of May 18 he struck his head against a tree or telegraph pole, and that when he came home he developed a headache, and soon afterward vomited. His appetite became poor, the vomiting continued, and about three weeks before his admission he noticed that he could not see distinctly, especially where near objects were concerned. His sight grew progressively worse. During his stay in the hospital his vomiting was very persistent, he developed deafness, and later almost complete blindness, being in a stupor most of the time. Examination of the eyegrounds was negative; the spinal fluid, examined on a number of occasions, was clear, and contained from 1 to 3 lymphocytes per cubic millimeter. Wassermann's reaction and Lange's colloidal gold tests were both negative, nor were tubercle bacilli found.

Examination of the blood showed about 7000 leucocytes, of which 75 per cent were polymorphonuclears. The blood Wassermann was negative. The Widal was negative. The urine was negative. The temperature was normal up to two days before his death, when it rose to 102°. The pulse ranged between 65 and 80.

At the autopsy, which I performed twelve hours postmortem, nothing of interest was found outside of the brain, with the exception of a terminal bronchopneumonia and of chronic tuberculosis of the right lung with old adhesive pleuritis.

With relation to the brain, the autopsy protocol states that when the dura mater was removed the brain surface was found to be very dry, and that a large quantity (about 100 cc.) of fluid escaped from the lateral and third ventricles during its removal. On examining the brain a tumorous mass, measuring 4.2 cm. anteroposteriorly, 3 cm. vertically, and 4.4 cm. transversely, was found in the region of the pineal gland, compressing the optic thalami (fig. 1), cerebellum, and corpora quadrigemina. The mass appeared slightly cystic, of a greenish-yellow color,

and had numerous hemorrhagic areas; a number of minute grayish areas were scattered through it. The brain, with the tumor in situ, was fixed in 10 per cent formalin for further study.

Examination of the formalin-fixed preparation showed that the tumor was somewhat nodular, and apparently attached to the pulvinar (figs. 2 and 3) of the left optic thalamus and to the region of the habenulae; the crura cerebri were markedly compressed

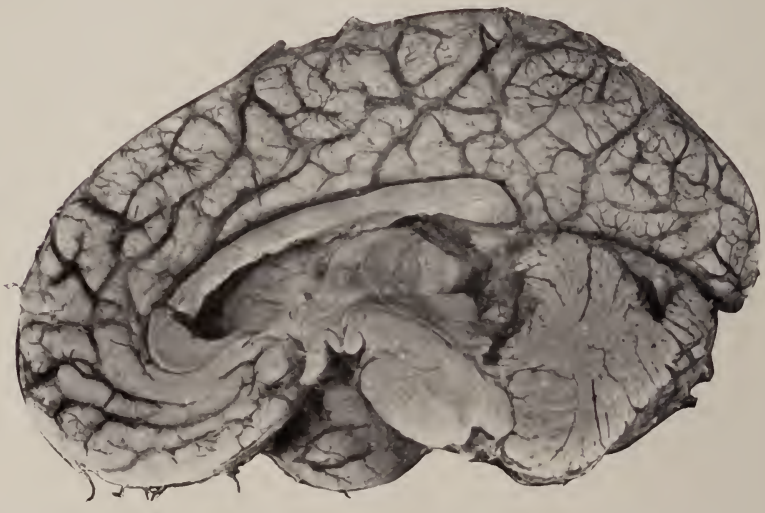

Fig. 1. Right half of brain, after removal of tumor, showing compression of thalamus, cerebellum, and cerebral peduncles. Note the elongation of aqueduct of Sylvius. The middle commissure is stretched and narrowed. The corpora quadrigemina are greatly displaced downwards.

and lengthened, the pons pushed somewhat downward. The central lobe of the cerebellum was compressed, forming a concavity, and the splenium of the corpus callosum was distinctly narrowed and pushed upward. The corpora quadrigemina were flattened, distorted, and displaced downward several centimeters over the anterior medullary velum. Saucer-like depressions were present in both optic thalami. On a median section of the brain,

the lateral ventricles were found dilated to several times the normal size (fig. 2); the third ventricle was also dilated, and the aqueduct of Sylvius compressed and greatly lengthened. The middle commissures were pushed forward, considerably elongated, and narrowed, while the posterior commissure was stretched and pushed downward.

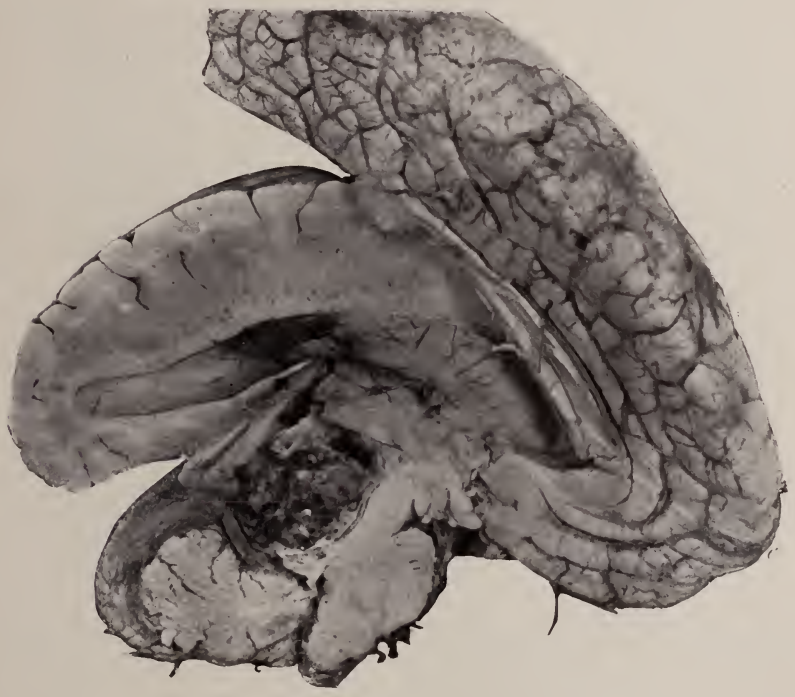

FIG. 2. Left half of brain, showing attachment of tumor to optic thalamus. The lateral ventricle is dilated.

The cut surface of the tumor was mottled, showing numerous hemorrhagic areas; a few small cysts were present, and many minute, light grayish-blue, translucent areas were scattered throughout the mass. These were firm nodules and resembled hyaline cartilage. At about the middle of the superior-posterior surface was a small tongue-like piece of tissue suggesting a

flattened epiphysis. The left antero-lateral one-third of the tumor was firmer, more homogeneous, and contained fewer hemorrhagic areas than the other portions; this was the region of attachment to the pulvinar.

Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Microscopically, the general plan of tissue arrangement is practically the same throughout the growth. There is a loose stroma of connective tissue, very embryonic in

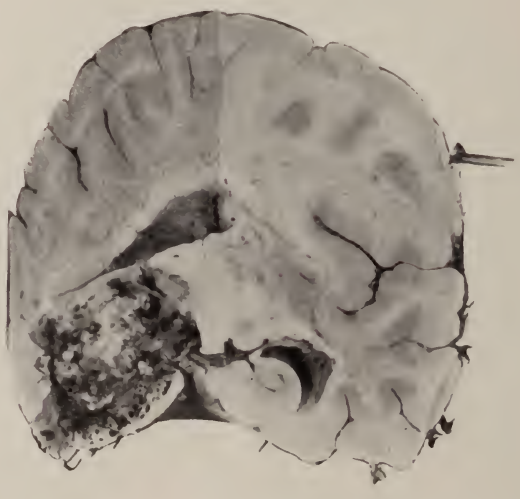

Fig. 3. Oblique coronal section of left half of brain through the tumor, showing its relation with the brain tissue.

character in some regions, in others of a more adult type but very cellular, while in still others the connective tissue fibrils are separated by precipitated serum, and only an occasional nucleus can be seen here and there. Hemorrhagic areas are numerous; within and around them are occasional endothelium like elements as well as connective tissue cells containing blood pigment. There is a large number of blood-vessels, the great majority being simple spaces lined with endothelium. Only a

few seem to be surrounded by a few irregularly placed muscle fibers.

Epithelium-lined spaces suggesting acini (fig. 4), ducts, and embryonic tubular viscera form a conspicuous portion of the histologic picture. As in Saxer's (10) case, the variety of shapes and sizes is almost beyond description; many are lined by a single layer of more or less regular columnar epithelium, others

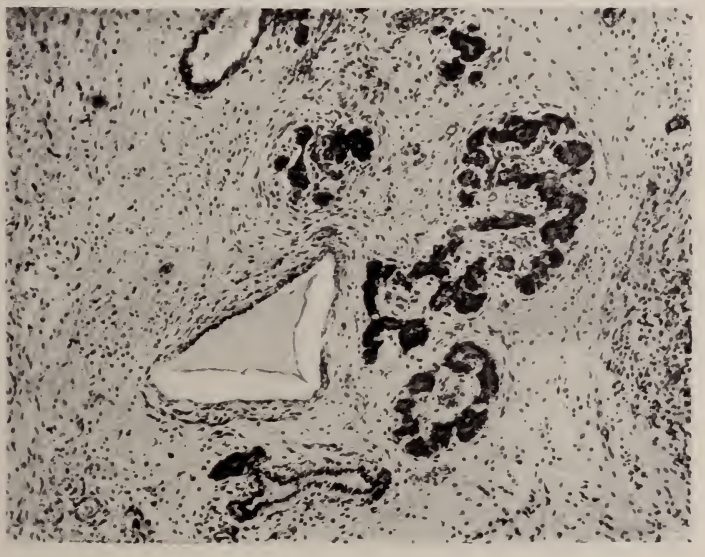

Fig. 4. Photomicrograph. Gland-like structures suggesting salivary glands. Below, a short portion of duct connected with a gland.

by flat cuboidal, and still others by an irregular multilayered epithelium (fig. 5). Typical stratified squamous epithelium is present in solid cords and as a lining to irregular spaces. This shows definite cornification, with the formation of very characteristic epithelial pearls (fig. 6). A number of these epithelial pearls situated in the center of narrow cords of epithelium very strongly suggest corpora amylacea.

Certain groups of small acinous gland-like structures resemble

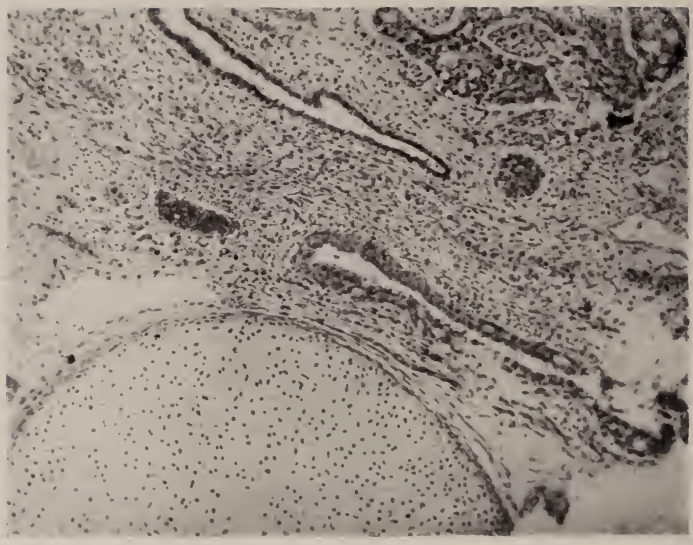

FIG. 5. Photomicrograph. Several spaces lined with irregularly arranged squamous epithelium. Below, a nest of fairly adult cartilage.

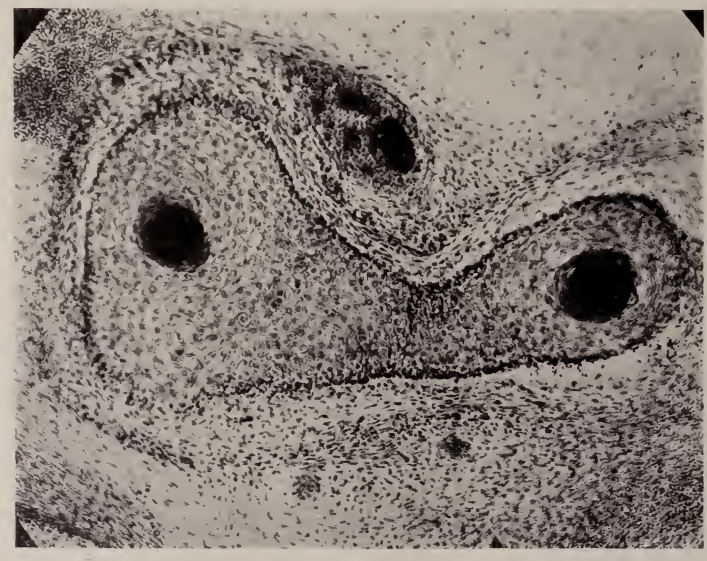

FIG. 6. Photomicrograph. Nest of stratified squamous epithelium showing cornified epithelium with formation of epithelial pearls.

salivary glands (fig. 4). The individual acini present minute lumina surrounded by four or five ill-defined, high cuboidal epithelial cells having a granular, faintly eosin-staining cytoplasm; the nuclei are large and basal in position. Many groups are associated with branched duct-like structures possessing distinct lumina and lined with a regular cuboidal epithelium.

There is a number of lumina lined with atypical columnar epithelium which strongly suggest primitive intestinal tract (fig.

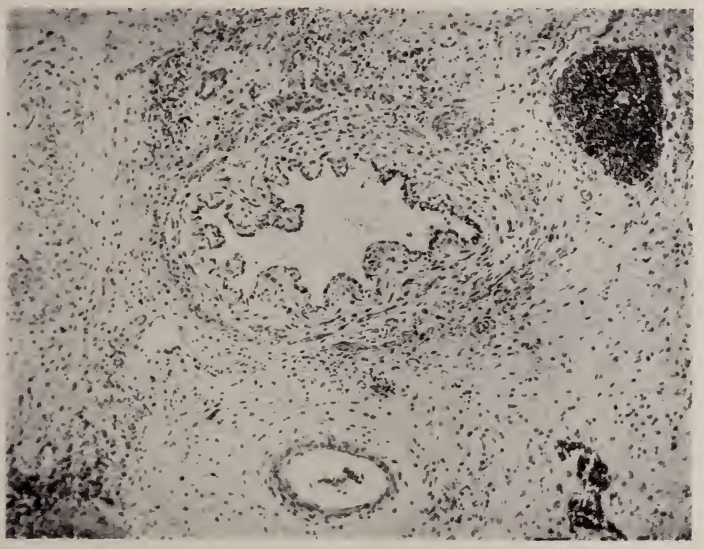

Fig. 7. Photomicrograph. Lumen lined with irregular columnar epithelium and surrounded by nonstriated muscle fibers.

7); these are surrounded by bundles of nonstriated muscle fibers. A few large spaces, lined with a high columnar epithelium, contain numerous more or less typical goblet cells (fig. 8). These are most probably entodermic structures. There are a few nests and cords of sharply defined, stratified squamous epithelial cells having hyalinized cores which suggest hair follicles.

A very striking feature in all the sections is the abundance of cartilaginous tissue. There are small round areas of a very embryonic type of cartilage, semimucoid in character (fig. 9).

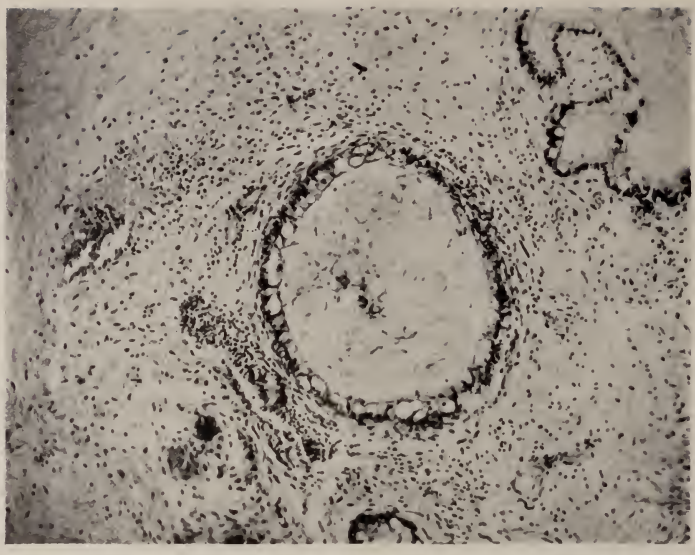

FIG. 8. Photomicrograph. Lumen lined with columnar epithelium containing goblet cells.

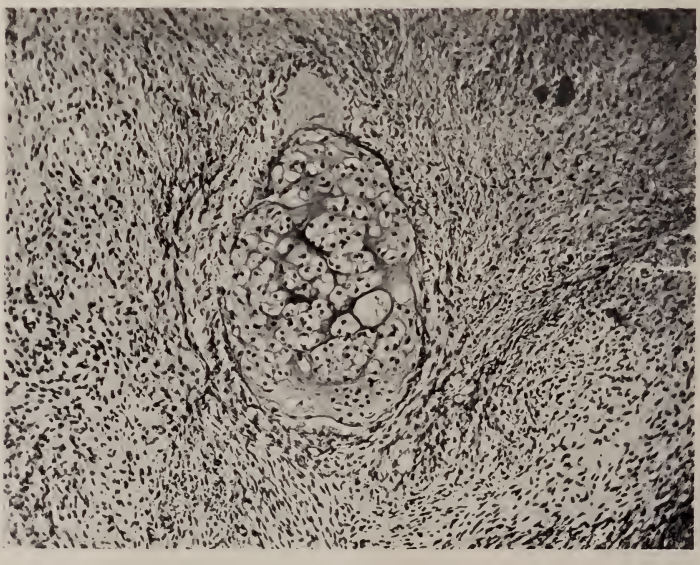

FIG. 9. Photomicrograph. Nest of embryonic cartilage surrounded by dense connective tissue.

Some areas have a dense bluish-staining hyaline matrix in which are embedded the characteristic cartilaginous cells (fig. 5). Then there are structures which, in the matter of differentiation, lie between these two. All the areas are definitely circumscribed, and none show any transformation into bone. Only one long spicule of bone was found in the sections examined (fig. 10); this is composed of several irregular lamellae separated by loose cellular connective tissue; the matrix is dense, containing some

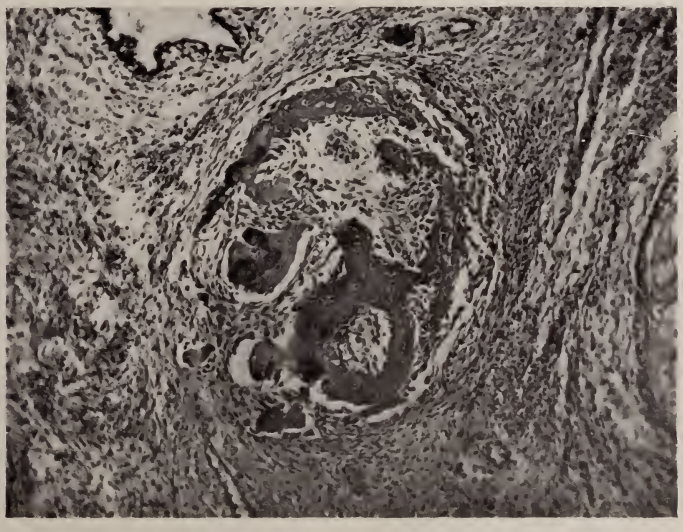

Fig. 10. Photomicrograph. Bone lamellae.

lime salts, and in the lacunae are the characteristic bone cells. This bone is surrounded by a dense zone of spindle-shaped connective tissue cells. No true bone marrow was found.

In one portion of the tumor there is a large area of irregularly arranged bundles of striated muscle fibers (fig. 11), in which the individual fibers run parallel and are separated by loose connective tissue. The majority of the fibers show only longitudinal striations, but a fairly large number show the cross striations just as distinctly as any well differentiated skeletal muscle fiber (fig. 12).

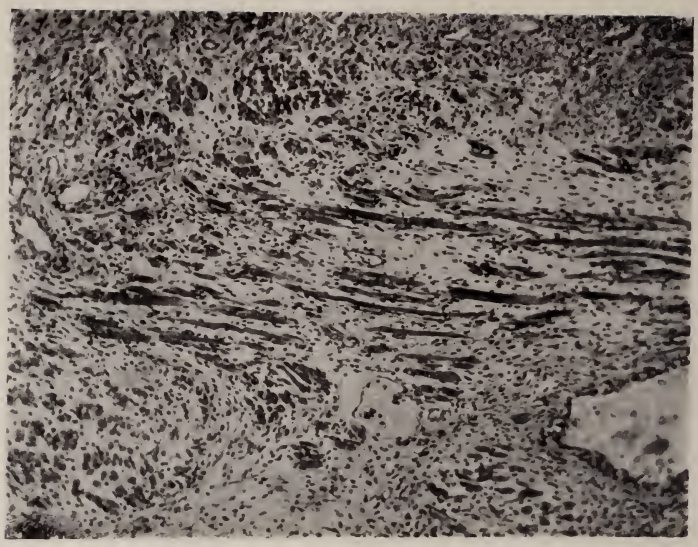

FIG. 11. Photomicrograph. Longitudinal and transverse sections of bundles of striated muscle fibers.

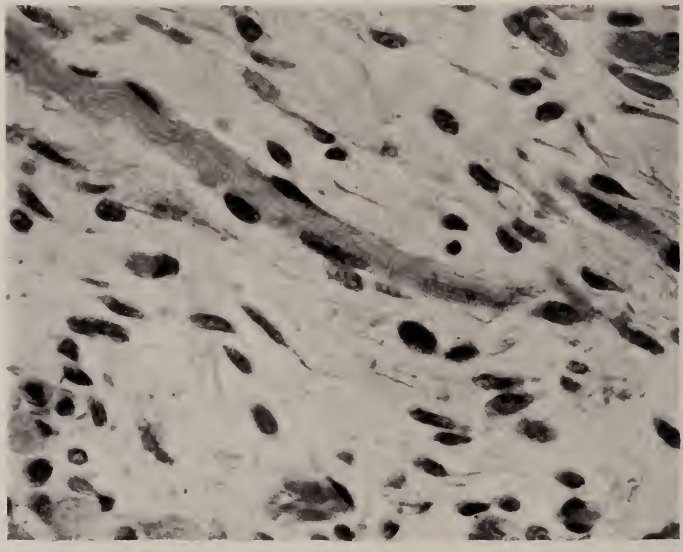

FIG. 12. Photomicrograph. Higher power photograph from figure 11, showing a single muscle fiber with longitudinal and transverse striations.

Careful search for nervous elements revealed in one area a small group of very large, densely staining cells with several processes. These resemble ganglionic cells but are not entirely convincing (fig. 13).

Throughout the entire tumor only an occasional mitotic figure was found in the more embryonic connective tissue.

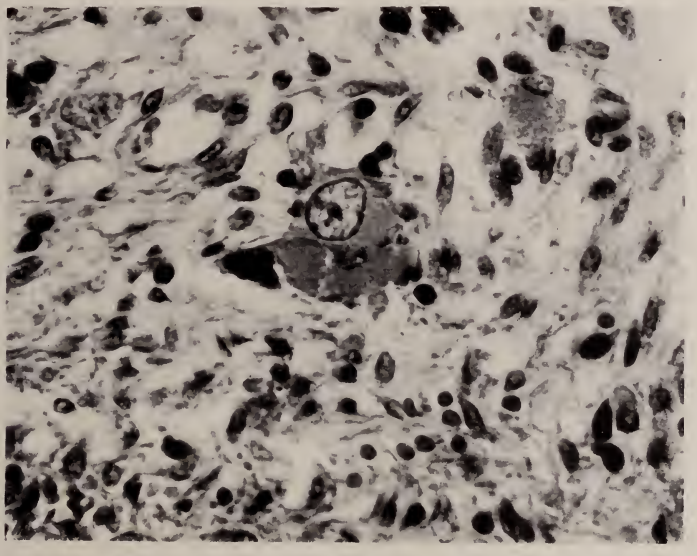

FIG. 13. Photomicrograph. High power photograph of large polygonal cell with processes, having distinct nucleus with nucleolus and strongly suggesting a ganglionic nerve cell.

Sections through the attachments of the tumor to the brain show a rapid transition from the fairly normal tissue, through atrophied and degenerating brain tissue, into the tumor tissue above described. In this proximal portion of the tumor, there is a greater abundance of cartilage and of stratified squamous epithelium showing cornification, and less numerous areas of hemorrhage, than in others. No pineal tissue was found in any part of the tumor; none was present in the previously noted tongue-like structure on the upper surface of the tumor mass.

The tumor just described contains elements which could have

arisen only from totipotential cells. There are definite ectodermal derivatives such as cornifying squamous epithelium, glands, probable hair follicles, and nerve cells; definite mesodermic structures, such as cartilage, bone, striated and nonstriated muscle fibers, and connective tissue; finally, the tubular structures suggesting intestinal tract, and those lined by columnar epithelium containing goblet cells, are most probably entodermal derivatives. Thus all the three primary germ layers are represented. Such a tumor cannot very well be explained by cellular inclusions from the scalp and calvarium during the later stages of development. Gutzeit (18), for example, had difficulty in explaining even the abundance of cartilage in these growths, since the bone in the calvarium is laid down without preceding cartilage formation. Neumann's (19) suggestion that the periosteum can produce cartilage during callus formation in membranous bone may help to eliminate the above difficulty from the explanation as to the origin of such a tumor from a late cellular inclusion; but how can the presence of the entodermal structures be satisfactorily accounted for by cellular inclusions arising during the development of scalp and calvarium? Askanazy's case (12) of primary chorionepithelioma of the pineal gland in a male presents still greater difficulties in the explanation of its origin, unless the hypothesis so strongly emphasized by Askanazy himself be accepted. This hypothesis, namely, that these tumors arise from the inclusion of totipotential cells before the gastrula stage, helps to unify the entire class of tumors in so far as their origin is concerned.

A study of the cases of brain teratomata discussed in the literature shows that tissues of mesodermic origin are the elements most commonly found in these tumors. However, excluding the present instance, there are only four examples (Strassmann and Streckner [8], Saxer [10], Gutzeit [18], and Neumann [19]) in which there is reported the presence of striated muscle fibers. Similarly in only one other case (Saxer [10]) is there described the presence of spaces lined with columnar epithelium containing goblet cells. Indeed, Saxer's resembles the present

case more closely than any other. It is interesting to note that practically all the cases which developed symptoms from these tumors died before the twentieth year.

Early embryonal inclusions of totipotential cells may be of frequent occurrence, but these, unless they have the proper conditions and environment, may degenerate before they actually "infect" the host. Certain tissues or organs, however, like the ovaries, for example, may favor the growth and development of such inclusions into tumors.

## CONCLUSIONS

1. Teratomata of the brain are very rare. When present, they most frequently originate in the pineal gland.
2. The origin of such tumors is best explained by the inclusion of totipotential cells very early in the development of the ovum.
3. The tumor here described presents derivatives from all three primitive germ layers.
4. This tumor belongs to the embryonal type of teratomata originating most probably in the epiphysis.
5. Only a very few brain teratomata described in the literature possess such a multiplicity of structures, especially structures like the striated muscle fibers and the goblet cells.
6. These tumors, if they develop so as to produce symptoms, generally cause death before the twentieth year.

I wish to thank Dr. E. M. Hammes for the opportunity of studying this case.

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# FURTHER INVESTIGATIONS ON THE HEREDITARY TRANSMISSION OF THE DIFFERENCES IN SUSCEPTIBILITY TO THE GROWTH OF TRANSPLANTED TUMORS IN VARIOUS STRAINS OF MICE

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## I. ON THE CAUSE OF DIFFERENCES IN SUSCEPTIBILITY TO THE GROWTH OF TRANSPLANTED TUMORS IN VARIOUS STRAINS OF MICE

It is generally admitted that differences in susceptibility to the growth of a certain tumor in animals belonging to different species and varieties are based on constitutional differences in these animals. This explanation is offered for the fact that a mouse tumor can not grow for any length of time in a rat, and for the still more striking fact that a tumor found in a common white mouse will not grow in a wild grey mouse or in a waltzing mouse. Michaelis, Haaland, and others, have shown, however, that even among animals of the same kind, different strains and families, which structurally appear to be identical, may differ markedly in their susceptibility to the growth of the same tumor. A number of investigators are inclined to attribute this latter difference not so much to constitutional differences hereditarily transmitted in certain families, as to external conditions, especially diet. They believe that this susceptibility can be changed experimentally.

Thus Haaland[1] found that a sarcoma which had developed in a mouse in Frankfort grew readily in mice bred in Frankfort and in some other parts of Germany, but grew only poorly in Nor-

[1] Berl. klin. Wchnschr., 1907, xliv, 713.

wegian white mice. He observed, however, that a few Frankfort mice, after they had been kept in Christiania for several months, also proved insusceptible to the mouse sarcoma, and he expressed the opinion that a change in the diet was responsible for this effect, inasmuch as in Christiania the animals received more bread and less protein (milk) than they had in Frankfort.

This explanation, however, is not founded on facts. There are no experimental data to exclude the possibility that those particular mice which had been transferred to Christiania might have represented one or more pure lines with a lower susceptibility to the implantation of tumors. We cannot be sure that they did not have less than the average susceptibility even at the time when they left Germany.

Equally inconclusive are the experiments of Stahr.[2] This author states that nobody seriously assumes that mice differ in their racial characteristics, and he points out that Gierke also holds the view that the conditions of life are probably the determining factors. He cites Haaland's observation in support of this interpretation. Like Haaland, Stahr finds differences in the susceptibility of mice obtained in Duesseldorf and in Berlin, and he attributes these variations, which later disappeared, to differences in diet. His observations, however, are altogether inconclusive. In view of the relatively slight differences which he found between the two strains, the number of mice used by him was too small to establish this contention.

It is of course true that the growth of tumors is dependent upon the supply of those substances necessary for tumor growth and for tissue growth in general, but the substances needed are essentially the same, whether the carcinoma develops in a German or American mouse; and it is therefore impossible to explain on this basis the specific adaptation of the carcinoma spontaneously developed in German mice to the particular soil in which it has arisen, and the similar adaptation of a carcinoma developed in American mice to its particular soil. Here we are dealing with constitutional differences between the cells and body fluids of

[2] Centralbl. f. allg. Path., 1909, xx, 628.

various strains of mice morphologically indistinguishable—differences which are not a direct function of the variability of external factors—although the constitutional differences may, perhaps, originally have been caused by external factors and may, under certain conditions, still be modifiable through external agencies.

Our own observations show conclusively that these differences are inherited, and not directly due to external conditions, since we have found that animals kept under identical external conditions may maintain such differences through a series of generations. We used for our experiments a carcinoma, No. IX, found originally in a Granby mouse, and growing in approximately 80 per cent to 90 per cent of mice bred in Granby. On several occasions mice were imported from Europe. These various strains were bred separately, but were kept under the same conditions as the original Granby mice. The No. IX carcinoma was inoculated into different generations of these various strains over a period of several years. The first strain to be imported we call "I European," the second, imported soon afterwards, "German" (or "II European"), while strains imported somewhat later are described as "Heitler" and "London."

The earlier inoculations extended through a period beginning in 1910 and ending in the spring of 1912. The later inoculations all began in April, 1912, and ended in September, 1914, with the exception of the "German" strain, in which they came to an end in March, 1914.

In the case of strains "European" and "German," the results of our earlier inoculations (old figures) are compared with those of our later transplantations; in strains "Heitler" and "London," the inoculations correspond to the later inoculations of the "European" and "German" strains. The old figures, which were published in our previous paper, are here reproduced in order to permit a comparison with the later results.

The results of the experiments are shown in Table I. The figures given under "continuous growth" indicate the percentage of definitely growing, not retrogressing tumors, while the figures for "takes" indicate the number of tumors that began to

grow, but later retrogressed. Inoculated grafts are considered to have "taken," if seven to ten days after transplantation a nodule could be felt at least as large as the fragment inoculated. In most cases these tumors continued to grow either indefinitely or at least for from six to ten days. Retrogressions usually became noticeable fourteen to eighteen days after inoculation, setting in at a time when the diameter of the tumors varied between a few millimeters and 1 to 2 cm.

In comparing the results of earlier with those of later inoculations in the "German" (II European mice) strain, we find similar figures. Throughout the various generations the percentage of definitely growing tumors remained very low (4–5 per cent on the average), and the variations were within the range of chance variations, the same relative constancy was found in the number of "takes."

The "Heitler" and "London" strains, which were obtained from Europe at a somewhat later date, both show a somewhat higher number of takes than the "German" strain, and the "Heitler" a somewhat higher percentage than the "London." In the six or seven generations during which the mice were observed, no increase in the percentage of definitely growing tumors took place; the average remained between 35 per cent and 41 per cent in the case of the "Heitler," and between 18 per cent and 25 per cent in the case of the "London" strain. The number of "takes" also remained constant. Nothing approximating the susceptibility of the American mice for tumor No. IX was observed. In all three strains the number of "takes" was similar to that among the American mice—though slightly lower—and the main difference between the American and the various imported mice depended on the number of retrogressions after an initial growth. The number of retrogressions was greatest in the case of the "German," and least in the case of the "Heitler" mice.

If we compare the rate of growth in the "Heitler" and "London" strains with the rate of growth in the American mice, no noticeable difference is found. The same applies to the "European" strain, which we shall discuss separately. Those tumors that grow definitely find approximately the same conditions in

all four strains. These strains, therefore, differ essentially in regard to the number of mice which permit a continued growth, or in regard to the number of mice which cause retrogression after a temporary growth. While the "Heitler" and "London" mice had a greater number of definitely growing tumors than the "European" and "German," those tumors that did retrogress receded at about the same period as in the latter two strains, and without showing a more active growth than the others during the period preceding retrogression. This fact implies that in those animals of these various strains which are not suited for definite growth, the conditions are about equal, and that these four strains differ essentially in the number of mice suitable for definitely growing tumors.

A different result is apparently obtained in the case of the first strain imported ("I European"). Here we noticed in the earlier series of inoculations an increase in $F_4$ to 30 per cent, while in $F_5$ the percentage of definite growths increased to 100 per cent; in the later series, the increase apparently begins in $F_3$ and is gradually augmented until $F_7$ is reached, attaining in this generation approximately the same percentage of definitely growing tumors as in the American mice. The number of "takes" seems to be somewhat higher throughout in "I European" than in the other imported strains.

The results obtained in the case of the "I European" strain are therefore apparently contradictory to those obtained with the others. In the case of the "I European" strain, a gradual change seems to have taken place which makes its behavior, so far as susceptibility to the growth of tumor No. IX is concerned, similar to that of the American mice; apparently, therefore, an adaptation has taken place in this strain to the new environmental conditions. A closer analysis shows, however, that these facts in all probability are to be interpreted in a different manner. With the beginning of the second series of inoculations (April, 1912), much sickness appeared among the "I European" mice, and the mortality rose to a high point. One family, however, called the "trio," consisting of two females and one male, had produced offspring that withstood the disease and gradually furnished the mice used for inoculation in this strain.

In $F_2$ of the second inoculation, among 30 descendants of the original mixed lot there were 7 per cent of definitely growing tumors, while the tumors grew in every one of the twelve descendants of the trio. In seven mice of the $F_3$ generation, descended from the trio, 86 per cent of the tumors grew definitely, while in the remaining forty-seven mice—the offspring of the mixed lot—only 32 per cent of the tumors grew definitely. In $F_4$ all, or the majority of the mice, were derived from the trio, and from $F_4$ on, all mice used for inoculation were descendants of the trio. The increase in the susceptibility of the mice corresponds, therefore, to the selection of the offspring of a certain family in the "I European" mice, which were, as it appears, a much better soil for the growth of tumor No. IX.

Evidently the "I European" strain comprises a number of pure lines, some of which were more favorable to the growth of tumor No. IX than others; and the gradual increase in the number of definitely growing tumors does not depend upon adaptation of the imported mice to their new environment, an adaptation entailing concomitant chemical changes, but rather on the selection of a pure line which was, from the beginning, more favorable to the growth of tumor No. IX. This interpretation is in accord with the experiments of Cuénot and Mercier,[3] who found that a strain of mice can be separated into several pure lines, in which the percentage of successful inoculations remains constant in successive generations.

## II. THE GROWTH OF INOCULATED TUMORS IN HYBRIDS BETWEEN EUROPEAN AND AMERICAN MICE

In an earlier paper[4] we reported on the inoculability of crosses between American and imported European mice; the latter corresponded to the "European"—("I European") and to the "German"—("II European") mice of our present and of our previous communications. Our tumor No. IX, which originated in an American mouse, was used for inoculation. We found that the

[3] Compt. rend. Acad. d. Sc., 1910, cl, 1443.

[4] Centralbl. f. Bakteriol., Erste Abt., Orig., 1913, lxvii, 135.

first generation of both kinds of hybrids was almost as favorable to the growth of inoculated tumors as the American mice, while in the second generation a marked fall in the number of definitely growing tumors took place; in the third generation, the fall was still more pronounced. So far, our results were similar to those obtained by Tyzzer, who crossed waltzing mice and white mice and inoculated their offspring with a tumor originally found in a waltzing mouse. Continuing our inoculations, however, we found a decided increase in the fourth and fifth generations. In interpreting our results, we assumed the existence of multiple factors as determiners of this susceptibility of certain strains to the growth of a certain tumor. The experiments were continued, the same tumor being inoculated into further crosses of "I European" and "German" with American mice. Our results are presented in Table 2, in which the figures obtained in earlier experiments are given side by side with those of more recent investigations.

Considering first the crosses of "I European" strain with American mice, these hybrids may be divided into one lot in which the original mixed "I European" was used for hybridization, and a second, in which the offspring of the European Trio were used. While the old figures were based on experiments carried out from December 1910 to March 1912, the later experiments were done during the period from March 1912 until September 1914. In the second and third sections respectively, the results of the inoculations are given. It will be noticed that, just as the offspring of the trio were a much more favorable soil for the growth of tumor No. IX than those of the mixed "I European" mice, so the percentage of progressively growing tumors was much higher throughout in the case of those hybrids into which the offspring of the trio had entered. But at the same time it is found that the peculiar curve representing the percentage of definitely growing tumors, which was observed in the first series, is no longer present. From $F_2$ to $F_5$ in the hybrids of the mixed "I European," as well as in the $F_2$ to $F_7$ generations of hybrids of the trio, the percentage remains almost the same throughout the various generations.

The same observation holds true in the case of the hybrids between the "German" strain and American mice. Here again the new series (extending over a period from March, 1912, to September, 1914) from $F_2$ to $F_5$ lacks the typical curve which we noticed in the first series. The latter had been done during the period from December, 1910 to March, 1912. Instead, we find an almost constant percentage of definitely growing tumors and of takes in the various generations, and the figures are very similar to those obtained in the case of the "European" hybrids. The fact that in both kinds of hybrids the same results were obtained makes it improbable that the results are accidental. But whether we have to deal in this case with a real adaptation of the hybrids to a new condition, or whether again a selection has taken place, or whether some other, hitherto unrecognized factor is responsible for these results, we are at present unable to state.

### III. THE GROWTH OF A CARCINOMA OF THE WHITE MOUSE IN HYBRIDS OF GREY AND WHITE MICE

Loeb found that a rat sarcoma could be transplanted into a hybrid between the grey wild and the white rat, and Jensen, also, has recorded a similar experiment. But definite figures indicating the frequency with which such transplantations succeed have, so far as we are aware, not yet been published.

A number of years ago Miss A. E. C. Lathrop obtained two wild grey mice, one from Michigan and the other from Vermont, which she bred with the strain "English," the Michigan mouse with the substrain "101" and the Vermont mouse with the offspring of "English Sable." Previous experiments indicate that carcinomata of the white mouse can be transplanted only exceptionally, if at all, into grey mice. In the "English" strains, our tumor No. IX could be readily transplanted in 80 per cent to 90 per cent, and it was of interest, therefore, to establish the frequency with which tumors will grow in hybrids.

The following Table 1 gives the results of such an experiment.

| | NUMBER OF MICE | CONTINUED GROWTH | TAKES |
|---|---|---|---|
| | | *per cent* | *per cent* |
| | *Michigan wild + 101* | | |
| $F_3$ | 24 animals | 32 | 65 |
| $F_4$ | 22 animals | 27 | 65 |
| | *Vermont wild + English Sable* | | |
| $F_2$ | 35 animals | 68 | 89 |
| $F_3$ | 51 animals | 55 | 90 |

These figures indicate that the conditions determining growth of a carcinoma of the white mouse in hybrids, are intermediate between those obtaining in wild grey mice on the one hand and in white mice on the other. In the "Vermont Wild" hybrids, however, the percentage of progressively growing tumors was approximately twice as high as in the "Michigan Wild" hybrids. Further, the figures for the "takes" are correspondingly higher in the "Vermont Wild" hybrids.

In the "Michigan Wild" hybrids, the figures for the $F_3$ and $F_4$ generations differ very little, and in the case of the "Vermont Wild" hybrids the figures for the $F_2$ generation are approximately the same as those for the $F_3$ generation. This indicates that here, also, there is not observed that curve of variation in the different generations which Tyzzer noticed in his hybridization between waltzing and common white mice, and which we ourselves observed in our first experiments with crosses between two strains of imported European and American mice. Both varieties of hybrids were timid and wild, resembling in this respect wild grey mice. The Michigan mice were chiefly grey, brown, or black, while the wild mice from Vermont showed all possible varieties of colors.

## SUMMARY

1. Differences in susceptibility to the growth of inoculated tumors observed in various strains of the same variety of animals are due to inheritable constitutional causes—to a specific adaptation of tumor cells to the soil in which they originated, and are not the expression of temporary changes in environment, par-

ticularly of the diet. An apparent exception to this rule in our experiments was due to the sorting out of a pure line that differed in susceptibility from the rest of the strain.

2. The typical variations in susceptibility of successive generations of hybrids to the growth of inoculated tumors, which were previously described by Tyzzer and ourselves, were not observed in the present series of experiments. The same typical variations were absent also in the successive generations of hybrids between wild mice and domesticated American mice.

TABLE 1

| Generation | I. EUROPEAN | | | | II. GERMAN | | | III. HEITLER | | IV. LONDON | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Continued growth | | Takes | | Continued growth | | Takes | Continued growth | Takes | Continued growth | Takes |
| | Old figure | New figure | Old figure | New figure | Old figure | New figure | | | | | |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| $F_1$ | 20 (64) | 17 (24) | 96 | 89 | 0 (37) | 25 (4) | 87 | 12 (8) | 63 | 22 (121) | 80 |
| $F_2$ | 14 (29) | 12½ (32) | 91 | 95 | 25 (4) | 0 (18) | 95 | 38 (42) | 88 | 26 (80) | 88 |
| $F_3$ | 7 (27) | 39 (54) | 92 | 95 | 0 (18) | 8 (12) | 89 | 41 (82) | 86 | 25 (92) | 74 |
| $F_4$ | 30 (39) | 51 (119) | 90 | 93 | 11 (9) | 6 (18) | 92 | 35 (57) | 86 | 21 (61) | 77 |
| $F_5$ | 100 (6) | 75 (156) | 100 | 90 | | 17 (6) | 83 | 39 (86) | 88 | 18 (80) | 81 |
| $F_6$ | | 76 (139) | | 93 | | 0 (2) | 50 | 38 (42) | 86 | 6 (13) | 75 |
| $F_7$ | | 86 (73) | | 97 | | | | 18 (11) | 82 | | |

The figures in brackets indicate the number of mice used in each experiment.

*Rate of growth*

| | 1 WK. | 2 WKS. | 3 WKS. | 4 WKS. | | 1 WK. | 2 WKS. | 3 WKS. | 4 WKS. | 1 WK. | 2 WKS. | 3 WKS. | 4 WKS. | 1 WK. | 2 WKS. | 3 WKS. | 4 WKS. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $F_1$ | 20 | 90 | 180 | 270 | | | | | | | | | | 25 | 80 | 180 | 260 |
| $F_2$ | 20 | 85 | 160 | 210 | | | | | | 15 | 70 | 150 | 230 | 20 | 75 | 160 | 230 |
| $F_3$ | 25 | 85 | 170 | 220 | | | | | | 20 | 70 | 175 | 240 | 20 | 80 | 140 | 240 |
| $F_4$ | 15 | 70 | 180 | 240 | | | | | | 20 | 70 | 155 | 210 | 25 | 60 | 130 | 200 |
| $F_5$ | 20 | 75 | 190 | 240 | | | | | | 25 | 75 | 160 | 240 | 20 | 75 | 155 | 210 |
| $F_6$ | 25 | 80 | 150 | 210 | | | | | | 20 | 80 | 160 | 230 | | | | |
| $F_7$ | 25 | 75 | 160 | 215 | | | | | | | | | | | | | |
| Normal rate of growth.. | | | | | | 20 | 75 | 155 | 220 | | | | | | | | |

TABLE 2

*First imported mice (European) plus American mice hybrids*

| | I. OLD FIGURES | | II. LATER FIGURES | | III. LATER FIGURES | | IV. LATER FIGURES | |
|---|---|---|---|---|---|---|---|---|
| | Continued growth | Takes | Crossed with original mixed European | | Crossed with the European trio | | Combined figures of I and II | |
| | | | Continued growth | Takes | Continued growth | Takes | Continued growth | Takes |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| $F_1$ | 68 (118) | 91 | | | | | 66 (12) | 85 |
| $F_2$ | 30 (118) | 89 | 41 (34) | 90 | 80 (49) | 92 | 65 (83) | 91 |
| $F_3$ | 24 (122) | 95 | 36 (34) | 86 | 78 (110) | 96 | 64 (144) | 90 |
| $F_4$ | 57 (143) | 96 | 45 (78) | 89 | 76 (75) | 89 | 59 (153) | 89 |
| $F_5$ | 59 (44) | 90 | 45 (94) | 93 | 65 (51) | 89 | 52 (145) | 91 |
| $F_6$ | | | | | 65 (74) | 97 | 65 (74) | 97 |
| $F_7$ | | | | | 73 (15) | 96 | 73 (15) | 96 |

*II. Imported mice (German) plus American mice hybrids*

| | 1. OLD FIGURES | | II. NEW FIGURES | | III. OLD AND NEW FIGURES COMBINED | | IV |
|---|---|---|---|---|---|---|---|
| | Continued growth | Takes | Continued growth | Takes | Continued growth | Takes | |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| $F_1$ | 100 (14) | 100 | | | | | |
| $F_2$ | 26 (54) | 89 | 41 (53) | 90 | 33 (107) | 88 | |
| $F_3$ | 2 (66) | 75 | 36 (90) | 92 | 21 (156) | 86 | |
| $F_4$ | 43 (123) | 92 | 40 (156) | 86 | 41 (282) | 89 | |
| $F_5$ | 51 (43) | 95 | 29 (56) | 91 | 39 (99) | 92 | |
| $F_6$ | | | | | | | |

The figures in brackets indicate the number of mice used in each experiment.

# PRECANCEROUS DERMATOSES

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The term "precancerous dermatoses" has that alluring quality which captivates the imagination and courts acceptance until analysis reveals that it is inappropriate. Its significance is scarcely clear. Does precancerous imply a state preceding an inevitable cancer, or one in which the possibility of cancer is latent and need not perforce gain expression? If so, what are the criteria by which we become cognizant of this potentiality? If the criteria clearly indicate early cancer, why precancerous; why is it not indeed cancer? At what point does precancerous lose its prefix? Is the problem one of medicine or etymology?

For more than a generation dermatologists have noted that epithelioma sometimes arises upon forerunning non-epitheliomatous lesions. Dubreuilh (1), twenty years since, was perhaps the first actually to catalogue such conditions. Here the matter rested until four years ago when Bowen (2) reported two cases, with definite clinical attributes, which he called precancerous upon the prophetic but inconclusive grounds that "as yet no signs of malignancy have appeared in these cases. It can hardly be doubted that such a sequel is imminent." In August, 1914, Darier (3) whose opinions command the utmost consideration, enriched this group by three, in a paper entitled "*La dermatose précancéreuse de Bowen.*" The very caption of the Parisian master's article implies an endorsement of Bowen's views, and this in spite of the fact that two of the three French examples of the disease were actually epitheliomata. At the conclusion of last year Bowen (4) published a sixth case and reviewed his first two and Darier's three. It has been vouchsafed me to increase this

number by one, a dermatosis corresponding both clinically and microscopically to that dealt with in Bowen's early contribution, and it is this case which has impelled me to study critically the entire question, with reference to Bowen's precancerous dermatosis in particular and the precancerous dermatoses in general.

It would be superfluous to transcribe in detail the histories of Darier's and Bowen's cases. Two were in women sixty-eight and thirty-nine years old; the other four were in men aged respectively forty-nine, fifty-two, sixty-five and sixty-one years. Two of Darier's, and Bowen's last patient, showed frank epithelioma, those of the former with regional glandular metastases. Clinically, the malady strongly suggested in arrangement the tuberoserpiginous syphiliderm progressing peripherally and cicatrizing centrally. At the advancing margin were numerous lesions varying in size from that of a dime to a quarter. They were discrete or confluent, gray, red, brownish or yellowish, and covered by greasy scales or crusts. Some were ulcerated and presented a slightly rolled margin. They had been present from four years, as in Bowen's second, to forty years, as in Darier's last case. The sites affected were variously the left buttocks (Bowen, Case I); outer surface of right calf (Bowen, Case II); left buttock, right axilla, right dorsal area, left groin (Darier, Case I); disseminated (Darier, Case II); right forearm (Darier, Case III); and disseminated over the trunk, back, and front (Bowen, Case III).

Histologically, all the lesions showed the following common features. Excepting where ulceration had taken place, the epidermis, papillary body, and corium were intact and showed certain fairly constant changes varying only in degree. In the epiderm, a markedly increased corneal layer consisting partly of nucleated and partly of unnucleated cells was found, and below this a granular layer varying in thickness. In the rete, there were two groups of alterations, a thickening with proliferation of the pegs, and a dyskeratosis. The thickening was due to a hyperplasia of malpighian cells, in evidence of which numerous regular mitoses were to be observed. As a result of this proliferation the pegs were lengthened, broadened, and distorted. In other words, a marked acanthosis existed. A certain degree of inter-

cellular edema with stretching of the bridges prevailed. Throughout the epiderm were found the dyskeratotic cells. These appeared as vacuoles twice to ten times the size of normal rete cells, and containing either a single, large, dark nucleus, or dividing nuclei from two to six in number. Some vacuoles, or dyskeratotic cells, presented a distinct outlining membrane. The largest of these structures were truly huge. In conformity to the outline of the pegs, the contour of the papillary body was altered and the papillae and corium down to the level of the subpapillary plexus were infiltrated. This infiltration tended to be sharply limited by a horizontal lower margin, and consisted of densely crowded lymphatic round cells with a rich admixture of Unna's plasma cells. A certain degree of proliferation of collagen and fibroblasts, with more or less vascular dilatation, shared in the picture. The lower levels of the corium were normal. In the ulcerating lesions, the usual changes of this process were found, the intact margins showing the alterations already enumerated. Darier's first and third, and Bowen's last case showed epithelioma in one of the many lesions examined in each instance, and in Darier's cases metastases had occurred in the regional lymph nodes. My own case may be briefly summarized as conforming clinically and histologically to those in Bowen's first paper. The patient was a man fifty-six years old, who for nine years had had an obstinate dermatosis on the right side of the neck, approximately over the mid area of the sternomastoid muscle. It resembled a crusted tubero-serpiginous syphilide, the convexity of which was emphasized by the presence of five or six scaling or crusted lesions, each a trifle smaller than a dime. They were dark brown, and removal of their covering revealed a proliferating area, moist with a serous secretion. All the histological features mentioned by Bowen were found, but there was no suggestion of epithelioma.

Of the seven instances of this malady, then, so far reported, three were malignant, four not. In the former group, in each instance, apparently only one of the numerous lesions was malignant, the others having escaped. We cannot reasonably apply the term precancerous to conditions in which recognizable epi-

thelioma exists. Thus there remains to be discussed in the four patients in whom the malignancy was absent, and in the three in whom it was present in only one of many lesions, what justification exists for so grave an assumption when the preponderance of evidence negates it. Is it the hyperkeratosis, the acanthosis, the dyskeratosis, the infiltration, or a combination of these factors which would urge such a conclusion?

The hyperkeratosis differs in no respect from that seen, for example, in certain forms of seborrhoea, lichen, ichthyosis, and the verrucous stages of numerous dermatoses, both congenital and acquired. Other examples might be added in great number. In general, the same applies to the acanthosis. Is it the dyskeratosis, then, which moulds our convictions? These peculiar vacuolated cells were first described by Darier in connection with the disease named for him and originally called psorospermosis. The structures in question were the psorosperms and were counted as parasites which provoked the disease. In the course of time, however, this view was discarded as erroneous, inasmuch as the psorosperms, or *corps rondes*, as the French called them, were found also in Paget's disease and occasionally in other dermatoses. They were finally recognized as an epidermal cell alteration, considered by Darier a dyskeratosis, and this author included among the dyskeratoses psorospermosis, now called keratosis follicularis, Paget's disease, and molluscum contagiosum. To these recently has been added Bowen's dermatosis. No one has included pointed condylomata, a characteristic feature of which are vacuolated epidermal cells with eccentric crescent-shaped nuclei, designated X-cells by Unna. These actually, however, have numerous points in common with Darier's *corps rondes*. There is clearly nothing cancerous about psorospermosis, molluscum, or pointed condylomata. The origin of the vacuolated cells in Paget's disease, as will be shown below, has perhaps nothing in common with the similar cells in other diseases. The molluscum bodies are by no means entirely identical with the so-called psorosperms, and it is likely that matters have been forced a trifle in associating the two so closely. It is undeniable, however, that the mere presence of dyskeratotic cells does not throw

the balance in favor of cancer. Conversely, too, although such structures are at times found in epithelioma, this is not frequent enough to be characteristic. In fact, they are usually absent, added evidence, indeed, against their significance in malignancy.

An infiltration of the variety seen in Bowen's disease is believed by Ribbert (5) to be an almost invariable concomitant of epithelioma, particularly at the onset. Still, no one would maintain that either the lymphatic type of round cell, or the plasma cell, is in any way pathognomonic; thus, there is nothing in the individual elements of the precancerous dermatosis to justify the use of the adjective. Nor does a scrutiny of their combination alter this conclusion. Many of the elements just enumerated are exhibited, for instance, in an ordinary wart, although the dyskeratosis and infiltration are lacking. A syphilitic primary lesion may show all the features enumerated except the vacuolated cells, and even a greater degree of acanthosis and more lymphocytes and plasma cells, while the same holds true of various other forms of inflammatory granuloma. In other words, the conclusion is inevitable that Bowen's precancerous dermatosis is a chronic inflammation possessing certain definite clinical and structural features. If this be true, how can the occasional association of epithelioma with this disease be explained? An answer will not be sought in vain in the wider field of what have been called precancerous dermatoses.

Darier (6) considers dermatoses precancerous when carcinoma so frequently originates from them as to exclude mere coincidence. Naevi; dystrophies such as senile keratoses, xeroderma pigmentosum, Roentgen dermatitis, and arsenical keratomas; leukoplakia; diverse dermatoses like lupus, scars, occupational skin diseases, dermoid cysts, ulcers, fistulae, inveterate psoriasis, and lupus erythematosus; and, finally, Paget's disease constitute this group. Ewing (7) reclassifies Darier's list under three heads, viz.; malformations, non-inflammatory degenerations, and inflammatory processes with their sequels. Although this adequately covers the field, not all senile keratoses are degenerations; a large number are inflammatory, arising on seborrhoeal soil, and seborrhoea is fundamentally an inflammation characterized by proliferation

of the horny layer and rete in which lie numerous edematous cells. The papillary body is infiltrated with lymphocytes and fibroblasts, the vessels are congested, and the fat content of the scale, germinative layer, coil glands, and vascular endothelium is increased. Such lesions in the aged assume many of the characteristics noted by Bowen in his dermatosis, while at all ages they may become the starting point of eczema. Senile skin itself, in weather beaten individuals, shows a degeneration in its elastic tissue; the fibers crumple, become short and wavy, take basic instead of acid dyes, or disappear entirely; hyalin degeneration of the collagen and atrophy take place, with proliferation of the epiderm and papillae. This is the nature of the second form of senile keratomas. They, too, have many of the features of seborrhoea.

It seems to me that this series of diseases might well be classified in the first place as congenital or acquired, and then further subdivided as follows:

A. Congenital:
   - I. Malformations—Naevi.
   - II. Dystrophies—Xeroderma pigmentosum.

B. Acquired:
   - I. Inflammations
     1. Hyperkeratoses
        - (a) Seborrhoeal keratomas.
        - (b) Inveterate psoriasis.
        - (c) Leukoplakia.
        - (d) Horns.
     2. Specific inflammations.
        - (a) Lupus vulgaris.
        - (b) Lupus erythematosus.
     3. General inflammations.
        - (a) Ulcers and fistulae.
   - II. Physical Agents.
     1. Exposure—Sailor's carcinoma.
     2. Actinic rays—Roentgen carcinoma.
   - III. Chemical agents—Arsenic, paraffin, soot.

IV. Regressive changes—Senile keratoma.
V. Malformations—Dermoid cysts.
VI. Scars from any cause, notably syphilis, lupus, and burns.
VII. Unclassified—Paget's disease.

Naevi represent the only congenital malformation which may lead to malignancy. It is chiefly the pigmentary forms which do so, but the process is so little understood and the eventuality so rare, that such a metamorphosis cannot be counted as an inherent property of moles. In comparison with the huge number of such defects observed, for scarcely an adult human being is without one or more, the number of melanomas is virtually negligible. Verrucous naevi even more rarely become malignant. Thus, it appears justifiable to eliminate birthmarks from the precancerous dermatoses. Xeroderma pigmentosum is a familial disease, the clinical features of which are well known. Microscopically, it bears a certain likeness to Roentgen dermatitis, and to sailor's and senile skin. Sooner or later the pigmented macules, keratotic lesions, and scaling surfaces develop into warts, ulcers, rodent ulcers, and other types of epithelioma, while Pollitzer, as Ormsby (8) reports, found carcinoma, sarcoma, and myxoma combined in one tumor. It appears more reasonable to class as precancerous the lesions of this disease than those of any other, since multiple epitheliomata inevitably develop. Even here, however, as will be seen, the term must be used with no little reserve.

Of the acquired variety, the commonest precursors of epithelioma are lupus scars, leukoplakia, seborrhoeal and senile keratomas, dermatitis caused by exposure, Roentgen rays, arsenic, paraffin, soot, cicatrices, and finally in a class of its own, Paget's disease. Of these the most important is the senile and seborrhoeal keratoma already described. Lupus vulgaris rarely is the site of malignancy. The majority of buccal and, in particular, lingual epitheliomata, arise from syphilitic cicatrices or leukoplakia; still, as compared with the large number of such alleged predisponents, this outcome is extremely rare. The same may be said of sailor's cancer; probably this disease is no more frequent

among the seafaring than among those who spend their life on land. Constant exposure to the Roentgen rays,as the early history of their employment illustrates, does actually predispose to malignancy with more regularity than any other condition, save xeroderma. Udo Wile (9) in a careful study of the subject, collected fifteen cases of carcinoma following the use of arsenic and developing upon arsenical keratoma, truly an infinitesimally small group considering the wide use of the drug. Pott's chimney sweeps' and Volkmann's paraffin workers' carcinoma are even better examples of this disparity. Ribbert (5, pp. 421–428) emphasizes the role of cicatrices, and among other citations includes Bergmann's views upon the causative influence of various inflammations and particularly of their end results, scars. He also notes von Brunn's statement to the effect that the majority of skin cancers arise from preceding inflammations. Hartzell (10) quotes figures from the Mission Dispensary in the Vale of Cashmere, where epithelioma is considered endemic. The inhabitants have a custom of carrying lighted braziers under their tunics; as a result, burns of the abdomen and thigh are frequent and are assumed to be the cause of subsequent malignant growths. Outside of the vale of Cashmere, however, untold people suffer similar injuries and countless scars result, but the number of epitheliomata developing upon them is trifling. In sum, this paragraph illustrates that cancer develops in skin which has previously been diseased or disturbed by common maladies, dystrophies, or chemical or physical traumas, the alleged exciting causes, however, being numerically out of all proportion in excess of the malignant changes they are supposed to produce.

True Paget's disease of the nipple is rare. Its connection with mammary carcinoma is admitted; its relation thereto, nevertheless, is still not entirely clear. The presence in the epidermis of vacuolated cells closely resembling those seen in Bowen's precancerous dermatosis stimulates speculation on the connection, if any exists, between these two maladies. Jacobeus (11) considers these structures as cancer cells which have wandered from the milk ducts and proliferated in the epidermis. Ribbert, (5, p. 250) and as he states, Hirschel and Aschoff, side with

Jacobeus. In general, however, this is not the accepted view, the majority of writers regarding the cells as dyskeratoses. Darier pointed out their similarity to psorosperms. Butlin (13), Thin (14), and Depage (Ribbert (5, p. 250), regarded the process as eczema. No one understanding the microscopic anatomy of eczema can for a moment take this view seriously. If anything lacks the earmarks of eczema, Paget's disease does. There are no vesicles, none of the characteristic inter- or intracellular edema, or scaling, or even the characteristic proliferation of the pegs. Neither, on the other hand, is there anything remotely suggestive of epithelioma in the skin of a case of Paget's disease. In the duct epithelium, however, one sees vacuolated cells identical with those seen in the epidermis. The most that can be positively stated of Paget's disease to-day is that a connection exists between the cutaneous changes and the glandular; but which is cause and which is effect, and what may be the significance of the skin manifestations, are all still unknown. Thus we have no reason to include this entity among the precancerous states. A number of writers have reported extramammary Paget's disease. Among these are Hartzell (15), who described one located on the forearm and associated with a naevo carcinoma; Morris (16) one on the neck; Crocker, Shields, Fox and MacLeod, Jungmann and Pollitzer, Ravogli, Dubreuilh, and finally, Fordyce. Dubreuilh's was on the vulva, Fox and MacLeod's on the umbilicus, Jungmann and Pollitzer's in the left axilla, Ravogli's on the nose, and Fordyce's on the buttocks. Of the eighteen cases assembled by Hartzell, nine were on or near the penis. Fordyce's (17) case deserves special mention, as he questions his own diagnosis in his title, and the disease actually proved to be rodent ulcer. In short, no good evidence exists of any of these cases having been Paget's disease, even though they were associated with cutaneous inflammation. Whether this constant dermatosis was cause or effect has been entirely overlooked by all of the writers, and there is just as much likelihood of the alleged eczemas having been post-cancerous as precancerous.

Of the conditions even less commonly and more remotely held

responsible for cutaneous cancers may be mentioned inveterate psoriasis, lupus erythematosus, which is a hyperkeratotic tuberculide, and chronic ulcers and fistulae. Chronic eczema, too, has been included, and recently Klausner (18) has reported an instance of lingual epithelioma following the buccal lesions of epidermolysis bullosa. He regards epidermolysis as a dyskeratosis, and in that manner accounts for its ability to excite carcinoma. It is quite apparent, however, that the association between malignancy and the dermatoses just enumerated cannot be very intimate, or the former would not be so extremely infrequent in connection with the latter. This is particularly the case with psoriasis, eczema, and erythematous lupus, which are among the commonest of skin diseases. Epidermolysis bullosa itself is very rare, and Klausner's case is unique, but it proves nothing except that two essentially different conditions existed in one individual.

Of all the conditions called precancerous, xeroderma pigmentosum is the only one which invariably is. Applied to the disease as a whole, the term is reasonable; as applied to given lesions, it is not. No one on earth is clairvoyant enough to select a given pigmented macule or scaling patch in a patient suffering with xeroderma, and point with certainty to its epitheliomatous future. And this, it seems to me, is the crux of the question. The principle here involved is applicable to all these dermatoses, including Bowen's. Given an epithelioma and viewing it in retrospect, both clinically and microscopically, we must admit that it was not cancerous before it became cancerous. At some stage in its evolution a point must have been reached nicely separating the two conditions, and yet uniting them, but by whom and by what methods was this to have been recognized? And ere this period was reached in the forerunner of the epithelioma, how was it to have been predicted? Clinical data alone could not suffice, neither could microscopical; for when the histological picture of epithelioma, however inconsiderable in extent, is once clear, the boundary separating the precancerous from the cancerous state has been passed.

It is obvious that after those epitheliomata which Cohnheim's

views suffice to explain, have been eliminated, the remainder must have evolved gradually and not according to the vague laws of predestination. As my old teacher, Dr. Prudden, epigramatically used to state, "there is no cell which suddenly exclaims, 'Lo, I shall be a cancer!" The process is insidious and undoubtedly governed by distinct impulses and inhibitions of growth in soil that is favorable. What these impulses are, and what this soil is, we have endeavored to determine before the full fledged neoplasm has become a fact; our concepts have been summed up in the word precancerous, and I believe that the word is a monument to the failure of our attempts.

One criterion is wanting which would at once set us aright. We lack a control, a standard of comparison. Until we possess figures showing how many cutaneous epitheliomata develop upon clinically normal skin, with which to compare those originating upon precancerous dermatoses, we cannot logically draw any deductions concerning the significance of the latter. As a matter of fact, if repetition be permissible, considering how common are senile and seborrhoeal warts, scars, and other skin disturbances supposed to predispose to cancer, and how infrequently they do so predispose, their conviction as precancerous appears to rest largely upon circumstantial evidence and the charge seems not wholly proved. It is my experience that more cutaneous epitheliomata develop without ascertainable forerunners than with them. Thus, of thirteen cases of which I have detailed records, ten arose upon normal skin, while one developed from a senile keratoma, one in a Roentgen worker, and one upon a leukoplakia of the tongue. If this should represent the actual ratio between malignancy developing without and with prodromata, the significance of the latter will cease to be regarded as very great.

And yet the fact remains that there is a group of lesions, the frequent end result of which is epithelioma. Is it wise and is it practical to call them precancerous? I think not, unless the term be applied to indicate the possibility without venturing the prophecy. Neither their gross nor their minute appearance is that of cancer, nor do they possess peculiarities so separating them from other lesions as to make it possible arbitrarily to con-

sider them the forerunners of cancer, as the microscopic similarity of Paget's disease and psorospermosis indicate. The term precancerous, however, creates a mental bias which facts do not justify and which is therefore confusing and scientifically impure. As Ewing (7) says, "The most serious argument against the theory of precancerous lesions is the fact that many carcinomas are not proven to be preceded by such changes." Among skin cancers this is peculiarly true, and yet it is more logical to presume that such lesions exist than that they do not, even though they be not clinically recognizable. The objection to the use of the term is, that without other than circumstantial criteria we cannot be sure of its correct application. In Bowen's precancerous dermatosis, as in the remainder of this long list of alleged precancerous conditions, all we know positively is that now and then, upon a given lesion, a cancer develops. We know that this happens even more frequently in skin which previously has appeared to be normal. Furthermore, there may be twenty other exactly similar lesions, as in Bowen's dermatosis and the senile keratomas, in which no malignancy develops, and hence these cannot be classed as precancerous. *Pre* means before and *precancerous* means before cancer. Thus, if exactly interpreted, and in science words must have exact meanings, precancerous dermatoses would be those which invariably become cancer. We know, however, that this is not a fact, and hence scientifically, as well as etymologically, the term precancerous dermatosis conveys a false impression. All that we actually do know is that a certain very small proportion of skin conditions which may be precancerous, ever prove to be so. For such a small number it is unjustifiable to create a special clinical group as Bowen attempts to do. This assertion is made in the full realization that cancer cannot spring from nowhere; that it must indeed possess a precancerous phase. It is maintained, however, that the precancerous stage is unrecognizable, both clinically and microscopically. Individual experience may justify the impression that such lesions are capable of leading to cancer, but this is vastly different from assuming that they will.

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# MODEL OF GASTRIC TUBULES IN EARLY GASTRIC CANCER[1]

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In the study of early gastric cancer in relation to gastric ulcer, I have previously reported[2] what appear to me to be segregated masses of gastric mucosa cut off from the surface by necrosis and subsequent formation of scar-tissue, and yet containing living epithelial cells apparently capable of functionating. Some doubt might well exist as to whether these were not misinterpretations of oblique sections. I long ago satisfied myself that this was not true by following throughout such isolated portions of tubules in serial sections. Of several such specimens I made photographs and of others drawings with the camera lucida covering the entire series. Last summer Mr. James A. Wynn reconstructed from the drawings of one such series a model, the photograph of which is herewith shown (fig. 1).

This reconstruction was made from drawings of sections through the edge of a large chronic gastric ulcer in one small area, on the opposite side of which there was unmistakable evidence of beginning cancer. The tubules bore the same relation to the normal surface of the mucosa that they here bear to the surface of the upper side of the case surrounding the model. It will be observed that a number of them have been completely

[1] Read by title before the American Association for Cancer Research, Washington, D. C., May 8, 1916.

[2] Wilson, L. B., The pathologic evidence of the relationship between gastric ulcer and gastric cancer. Collected Papers, Mayo Clinic, 1913, 149–159. Wilson, L. B. and McDowell, I. W., A further report of the pathologic evidence of the relationship of gastric ulcer and gastric carcinoma. Am. Jour. Med. Sciences, 1914, cxlviii, 796–816.

reconstructed and that they do not communicate with the surface. Some of the smaller ones contain atrophic epithelium. The larger ones are very much larger than the ends of other gastric tubules from a nearby normal region reconstructed on the same scale, and shown in the photograph. The epithelium of the dilated tubules was much swollen. Some of the tubules were cystic and filled with mucus.


FIG. 1. *A*, Reconstruction of group of portions of gastric tubules segregated in border of gastric ulcer: *1*, tubules; *2*, surface of cover representing base of ulcer. *B*, group of normal tubules from neighboring region in same specimen—same magnification as *A*.

Since the segregated glandular masses contained epithelium capable of functionating and still connected with their nerve and blood supplies, their possible relationship in cases of gastric ulcer to the pain occurring during digestion is suggested. Food in the stomach may cause the same sort of pain in these areas as is present in the parotid gland when a patient with mumps eats sour foods.

# PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

## NINTH ANNUAL MEETING

*Held in Washington May 8, 1916*

### 1. Report of the Council

The following members were present at this meeting: Dr. H. G. Wells, president; Dr. R. Weil, Dr. F. P. Gay, Dr. James Ewing, Dr. H. R. Gaylord, Dr. F. C. Wood.

The report of the treasurer, showing a balance on hand of $301.65, was read and accepted.

The election of a new councillor, Dr. J. B. Murphy of the Rockefeller Institute, took place, to replace Dr. S. B. Wolbach of Harvard University, automatically retired by the time limit.

The following officers were elected for the ensuing year: Dr. Harvey R. Gaylord, president; Dr. Francis C. Wood, vice-president; Dr. Richard Weil, secretary and treasurer.

The Council for the ensuing year consists of the above officers and Dr. F. P. Gay, Dr. James Ewing, Dr. H. G. Wells, and Dr. J. B. Murphy.

NEW MEMBERS

*Active members*

Dr. E. S. L'Esperance, New York
Dr. J. E. Sweet, Philadelphia, Pa.
Dr. M. T. Burrows, Baltimore, Md.
Dr. R. T. Frank, New York
Dr. E. T. Bell, Minneapolis, Minn.
Dr. Maud L. Menten, St. Louis, Mo.
Dr. Frederick Prime, New York
Dr. H. E. Robertson, Minneapolis, Minn.
Dr. J. A. P. Millet, Buffalo, N. Y.
Dr. H. M. Stevenson, New Rochelle, N. Y.
Dr. C. F. Burnam, Baltimore, Md.
Dr. Herbert Fox, Philadelphia, Pa.
Dr. M. J. Sittenfield, New York

*Associate members*

Dr. T. F. Cooke, Buffalo, N. Y.
Dr. S. R. Brown, New York.
Dr. O. F. Ormsby, Chicago, Ill.
Dr. Dean Lewis, Chicago, Ill.
Dr. J. P. Hoguet, New York
Dr. John F. Erdmann, New York
Dr. B. S. Barringer, New York
Dr. H. C. Taylor, New York
Dr. B. W. Bolling, New York
Dr. N. E. Brill, New York
Dr. A. E. Hertzler, Kansas City, Mo.
Dr. E. C. Franing, Galesburg, Ill.
Dr. W. T. Bovie, Boston, Mass.
Dr. Henry Lyman, Boston, Mass.
Dr. D. J. Glomset, Des Moines, Iowa
Dr. D. B. Phemister, Chicago, Ill.

Dr. C. C. Little of Boston and Dr. J. C. Bloodgood of Baltimore, were transferred from associate to active membership.

The resignations of the following members were accepted: Dr. Joseph Blake, Dr. Thomas Ordway, Dr. Horst Oertel, Dr. R. L. Thompson, Dr. A. M. Burgess.

The following resolution was passed:

That the Council approves of the securing of funds to constitute a reserve fund, and to be applied to the development of the Journal or to the furtherance of Cancer Research, at the discretion of the Council.

At the meeting of the Association on May 9, the following amendment to the Constitution was unanimously passed:

The annual dues of the Society for 1916, and for subsequent years, shall be five dollars ($5) per year, the increased dues entitling each member to a subscription to The Journal of Cancer Research.

### 2. Thyroid Tumor in the Sea Bass

*Mr. M. C. Marsh.* See Journal of Cancer Research, vol. 1, no. 1.

### 3. Spontaneous Sarcoma in the Mouse

*Miss Maud Slye, Miss Harriet F. Holmes, and Dr. H. G. Wells* (of Chicago):

SUMMARY

In a series of 12,000 mice of all ages, dying natural deaths and completely autopsied except as to the cranial cavity, 88 growths were found meeting all the criteria for sarcoma. We have rigidly excluded all doubtful lesions such as lymphoid growths corresponding exactly to lymphosarcomas in man and mediastinal lymphosarcomas. Of these 88 tumors, 46, or about half, were spindle-celled sarcomas, 12 were osteoid sarcomas, while the others were of various types. There were no melanosarcomas or typical myeloid giant-celled sarcomas.

The most common point of origin was in the subcutaneous tissue, a large proportion of the growths apparently arising in the mammary gland. Next in frequency were the bones, with 12 tumors. In at least eleven cases, the sarcoma arose at the site of, and subsequent to, an injury.

Metastasis occurred in 26 per cent of these tumors, the osteoid sarcomas giving 75 per cent of metastases, whereas the spindle-celled sarcomas showed only 6, with metastasis in 46 cases. Metastasis occurred most often in the lungs, next in the liver, and third in the lymph nodes.

In two cases, a sarcoma and a carcinoma existed side by side. One other tumor presented a structure which in some parts seemed to be carcinoma, other parts suggesting a sarcomatous structure. This resembled the carcinomas with sarcomatous transformation of the stroma that have been described in the literature, and is not included in the present series.

Two tumors arose in the mammary gland, structurally resembling the embryonal adenosarcomas of the kidney, although there was no involvement of the kidney in these mice. These also are not included among the 87 typical sarcomas. In one mouse there were two distinct independent sarcomas, and in four others this possibility could not be excluded. Twenty per cent of the sarcoma mice showed coexisting other tumors, of which tumors of the lung were most frequent.

In some of the liver metastases from osteosarcomas there appeared areas of fatty marrow tissue, apparently arising from and partly replacing the tumor tissue.

DISCUSSION

*Dr. E. R. LeCount* (Chicago): Dr. Wells was so kind as to let me look over these very interesting secondary growths in the liver, and, as nearly as I can interpret them, this paper appears to be a genuinely new addition to our knowledge of tumor growth.

Some years ago I had occasion to examine a sarcoma in the right tibia of a young woman, with extensive metastases in the chest; the secondary growths contained bone of varying sorts, but no cartilage. Apparently the secondary growths had been entirely produced by bone-making cells—that is, by osteoblasts. This is, as you may know, quite uncommon in secondary growths from bone sarcomas.[1]

But in these secondary growths of the liver we are apparently dealing with a new phase of secondary growths from primary malignant tumors of bone—secondary growths in which, after a period of residence and growth in the liver, what is finally represented is adipose tissue and other structures resembling bone-marrow. This explanation is largely supported by the observation of Dr. Wells, of beginning secondary growths containing both cartilage and bone-making cells, and other intermediate lesions on the way to bone-marrow.

So far as I know the literature in connection with bone tumors and sarcomas of bone in general, this observation is, as I have said, something genuinely new.

*Dr. James Ewing* (New York): This is a very peculiar observation that Dr. Wells has made in these livers, and he and Dr. LeCount have probably come to the right conclusion. At the same time, in order to convince me of their conclusion, certain difficulties will have to be cleared up.

Does Dr. LeCount believe that bone once existed in this liver and has been absorbed, leaving fatty material?

*Dr. LeCount*: Yes.

*Dr. Ewing:* That process is quite new to me. No doubt I shall be able to grasp it after due consideration, but at first sight it strikes me

[1] Johns Hopkins Hosp. Bull., 1909, xx, 361.

as somewhat improbable. As far as the production of bone-marrow is concerned, the only bone-marrow that I have seen in secondary growths, or in the calcification of organs, has been in particular locations made favorable for the development of bone-marrow by calcification. Bone-marrow does not form on the outskirts of fatty tumors, but only in the niches or spaces which reproduce the circulatory conditions common in normal marrow. In one of Dr. Wells' cases, there was a metastatic nodule with bone-marrow lying outside of it. This is new to my observation; and while I do not doubt that it is bone-marrow, it would appear to me at first sight like a lymphoid infiltration. I should like to see these tumors under the microscope before attempting to express any opinion about them.

*Dr. Leo Loeb* (St. Louis): Did I understand Dr. Wells to conclude that all sarcomas arising from carcinomas are due to a transformation of ordinary epithelial cells into epithelial cells that have a spindle shape; I can hardly assume such a conclusion to be correct. We know that the sarcomas secondarily produced differ in other important respects from carcinomas, and we have to deal here with much more fundamental differences than a mere change in the shape of cells. There can be no doubt that in most cases the sarcomas are made up of actively proliferating connective tissue cells that have been in contact with the carcinomatous area.

*Dr. H. R. Gaylord* (Buffalo): Dr. Wells' observation regarding the formation of bone in the tumor he has demonstrated, and the interpretation of the surrounding fatty tissue as bone-marrow, recalls the fact that in the osteo-chondrosarcoma of the chicken of Rous, implants of this tumor which undergo healing leave behind a sphere of laminated bone containing fully differentiated red bone-marrow. In this neoplasm, however, the relation of the bone shell to the enclosed bone-marrow is such as might be expected; like Dr. Ewing, however, I am a little confused by the idea of bone-marrow surrounding a central mass of calcified bone.

*Dr. E. E. Tyzzer* (Boston): It does not seem very remarkable to me to find bone-marrow in the liver of the mouse, for leucocytogenetic tissue in this organ is of very common occurrence in this species. The unusual feature appears to be the presence of the fat in association with marrow.

*Dr. W. H. Woglom* (New York): I should like to ask Dr. Wells whether any of these tumors he has shown could be referred to injury of any sort, or whether no such connection could be traced.

*Dr. Wells* (closing): I agree fully with everything Dr. Ewing has said. I recognize the fact that the marrow ought to be inside the bone and not outside, and that the explanation is not convincing. I

have simply advanced it because I have not been able to reach any other conclusion, and I hope somebody else here can advance a more reasonable solution.

The same thing, practically, applies to what Dr. Loeb has said. I do not insist on the nature of this particular tumor which resembled sarco-carcinoma, still, the employment of special stains has not established indubitably that it is a true sarco-carcinoma.

In reply to Dr. Woglom; of these mice there were eleven that showed a very specific relation to trauma; that is, a mouse would be injured in fighting, or by some accident, would be put into the cage where the injured mice are kept, and would later develop a sarcoma at the site of that injury. Of course, I cannot tell how many more mice of the forty-six had injuries, because the life of a mouse is beset by many accidents; but certainly in eleven the relation was very definite indeed.

### 4. Tumors in Relation to Senescence in Dogs

*Dr. E. W. Goodpasture (Peter Bent Brigham Hospital and Harvard Medical School, Boston):* The material for this study consists of the autopsy findings in fifty aged dogs. The animals were procured from an Animal Rescue League immediately after death, and were chosen solely because of such evidence of age as loss of teeth, the presence of cataract, decrepitude, etc. None was brought to the laboratory because of the presence of a tumor. The dogs averaged between eight and twelve years of age, this estimate being approximate, as accurate data could not be obtained. Only three animals thus chosen have shown no evidence of tumor formation in any organ or tissue; each of the fifty animals here described had multiple tumors either benign, or benign and malignant, in more than one organ. I have classified as malignant those tumors which were invading surrounding structures or had metastasized; in this group there have been placed thirteen tumors; Adrenal medulla (1); Carcinoma female breast (2); Myelogenous leukaemia with metastatic tumors in many organs and tissues (1); Carcinomata of liver (2); Carcinomata of testis (4); Carcinomata of lung (2); Carcinoma of submaxillary gland (1). In addition to these malignant tumors, there has been found a great variety of multiple benign adenomatous overgrowths in epithelial organs, lymphomata of the spleen, fibromata, lipomata, and haemangiomata in various tissues. Occurring hand in hand with these cell overgrowths are more or less characteristic cell degenerations in the organs and tissues in which the tumors arose. Both are dependant upon and characteristic of senescence in these animals.

There were thirteen females and thirty-seven males in this series. The following chart indicates the total number of tumors and the percentage occurrence in each organ, and the number and percentage of malignant tumors.

Twenty-six per cent of the animals showed malignant tumors.

| ORGANS | TUMORS BENIGN AND MALIGNANT | | | | | MALIGNANT TUMORS | PER CENT |
|---|---|---|---|---|---|---|---|
| | Male | Per cent | Female | Per cent | Total per cent | | |
| Adrenal | 29 | 78 | 13 | 100 | 84 | 1 | 2 |
| Breast | 0 | 0 | 4 | 30 | 8 | 2 | 4 |
| Blood vessels | 2 | 5 | 0 | 0 | 4 | | |
| Bone marrow | 1 | 3 | 0 | 0 | 2 | 1 | 2 |
| Gall bladder | 3 | 8 | 2 | 15 | 10 | | |
| Hypophysis | 1 | 3 | 0 | 0 | 2 | | |
| Liver | 32 | 86 | 9 | 70 | 82 | 2 | 4 |
| Lung | 1 | 3 | 2 | 15 | 6 | 2 | 4 |
| Ovary | | | 6 | 45 | 45 | | |
| Pancreas | 5 | 15 | 0 | 0 | 10 | | |
| Prostate | 22 | 66 | | | 66 | | |
| Skin | 5 | 15 | 2 | 15 | 14 | | |
| Spleen | 27 | 71 | 12 | 90 | 78 | | |
| Stomach | 4 | 12 | 1 | 7 | 10 | | |
| Subcutaneous tissue | 5 | 15 | 3 | 22 | 16 | | |
| Submaxillary | 3 | 9 | 0 | 0 | 6 | 1 | 2 |
| Testis | 21 | 63 | | | 63 | 4 | 8 |
| Thymus | 1 | 3 | 0 | 0 | 2 | | |
| Thyroid | 4 | 12 | 7 | 52 | 22 | | |

## 5. Notes on Some Experiments with the Rous Chicken Sarcoma

*Dr. B. T. Simpson* (Buffalo, N. Y.): The following notes have been collected as worthy of record from various experiments with the Rous chicken sarcoma.

We succeeded in obtaining positive transplantation with dry powdered tumor which had been kept one year and seven months.

Ground sarcoma exposed to 51 mgm. of radium bromide for twenty-four hours was transplantable, taking 100 per cent of inoculated fowls.

Freshly ground sarcoma subjected to the temperature of liquid air for forty minutes, gave positive results in 50 per cent of cases.

Freshly ground tumor mixed with 100 per cent glycerin and placed in the ice-box, remained viable for one hundred and fifty days. The percentage of successful inoculations was fifty, and the length of time before the appearance of the tumor was lengthened from twelve to twenty-two days.

Twenty pigeons exposed to massive doses of X-ray, using the Coolidge tube, were refractory to sarcoma.

DISCUSSION

*Dr. E. F. Smith* (Washington): How are these results to be reconciled with the findings of Dr. Murphy, who seemed to get opposite results? If I understand Dr. Murphy's work, he reduced the resistance by minimum exposure, while Dr. Simpson seems to have obtained a different result as a consequence of one intense treatment.

*Dr. H. R. Gaylord* (Buffalo): A word or two in explanation of Dr. Simpson's experiments. Our expectation was that in the period immediately following the exposure to x-ray, when the spleens were found to be markedly injured and greatly reduced in size, this condition would be associated with a susceptible state as regards implantation. At this period the lymphocytes were greatly reduced in number. As you have seen from Dr. Simpson's statements, this period is one of resistance, and in seeking for an explanation of these findings it has occurred to us that possibly the x-ray mobilizes certain factors in the splenolymphatic system, which, through their entry into the circulation, produce the transient resistant state he has observed. As Dr. Ewing has remarked, the entire animal is, of course, exposed, the destructive effects of such an exposure being seen in the low lymphocyte counts.

*Dr. James B. Murphy* (New York): There is one point which I think might explain Dr. Simpson's results. We have found that when massive doses of x-ray are given, the animal's general health is considerably affected. It is well known that tumors will not grow in such animals, regardless of the cause of the ill-health. We have considered this as an explanation of our results along this line which confirm those of Dr. Simpson. To obtain a maximum destruction of the lymphoid system with a minimum damage to other structures, we have found it necessary to use a split dose method, that is, a very small exposure repeated at daily intervals throughout from 6 to 10 days. By such a method, animals can be carried through with little or no general disturbance. This, therefore, will explain, in a way, Dr. Simpson's results, as his dose appears to have been very near the fatal dose as exhibited by one series of these animals.

*Dr. Smith*: What doses were given?

*Dr. Murphy:* The doses given were less than a quarter of the so-called erythema dose, repeated at daily intervals throughout from 6 to 10 days. The Coolidge tube was used at a distance of 25 cm. with 10 milliamperes of current and a 3-inch spark gap.

*Dr. Henry Schmitz* (Chicago): Dr. Cole has shown that the Coolidge tube develops practically one erythema dose per minute in the human being, and consequently fifty erythema doses must have been given in this experiment, which is an enormous amount. I believe that this has

probably something to do with the difference in the results obtained by the two observers. We should, by the interposition of a metal screen, exclude all those rays which could possibly strike the skin, and only allow those rays to pass which will penetrate into the deeper tissues. It is the portion of the x-ray or the radium that is absorbed, not the one that passes through the tissues, which does the work. I believe that if the experiments were carried on in such a way the results might be different, though of course, I offer this idea only as a suggestion.

*Dr. Gaylord:* I should like to lay emphasis upon a fact which Dr. Simpson has not emphasized, that later, when the spleens have undergone regeneration, a refractory state again supervenes, not as definite as the first, though fairly distinct nevertheless.

*Dr. Simpson* (closing): Dr. Murphy has answered Dr. Smith's question. Of course, this experiment is quite different from those of Dr. Murphy, who used very small doses at twenty-four hour periods. In our experiment, one massive dose only was given. We found that any dose, given after the initial one, would kill the mice; their vitality, therefore, must have been very low. Yet no ill effects were apparent externally after the one dose; the mice looked healthy, they had no burns, and there seemed to be nothing the matter with them except a tremendous blood fall, and a diminution of the size of the spleen with loss of lymphoid tissue.

### 6. Alkalinity of the Blood in Malignancy and Other Pathological Conditions

*Dr. Maud L. Menten* (St. Louis): Measurements of blood from a large number of individuals have revealed the fact that a definite relationship exists between the atmospheric pressure and the reaction of the blood. Where the atmospheric pressure is as high as 762 mm. of Hg. or more, the blood attains a value of pH. 7.72, almost its maximum alkalinity, and when the pressure falls to 730 mm. of Hg. or lower the acidity of the blood increases to pH. 7.40. This is due, possibly, to varying oxygen pressure in the alveoli of the lung, causing a greater or less absorption of oxygen with a corresponding variation in the amount of oxyhaemoglobin formed.

Weight is given to this hypothesis by the fact that the relationship can be expressed by a dissociation curve, analogous to that of haemoglobin. The atmospheric pressure, therefore, is such a dominant factor in determining the reaction of the blood that its influence completely masks any small differences which might arise from pathological conditions.

On this account, electrical measurements were made on sera containing minimal amounts of $CO_2$, from normal patients and from those suffering from cancer or other pathological conditions.

Briefly, these measurements showed that in sera from normal indi-

viduals, from those whose blood gave positive Wassermann reactions, and from cases of pregnancy, the reaction was between pH. 7.90 and pH. 7.94.

Sera from more than 60 patients with superficial malignant growths or with neoplasms of internal organs, showed an increased alkalinity varying from pH. 8.00 to pH. 8.44.

A few exceptions were noted, viz., two cases of carcinoma of the sigmoid flexure, one of which gave a normal reading and the other only a slightly increased alkaline reaction. Three cases of carcinoma of the lip also gave normal reactions, while three others showed an alkalinity lying between pH. 7.94 and pH. 8.00.

Among other pathological conditions in which increased alkalinity obtained, may be mentioned cholelithiasis and diabetes mellitus; in the latter case, the alkalinity became more marked after the Allen starvation treatment or the administration of $NaH\ CO_3$.

Increased acidity occurred in cases of tuberculosis, pemphigus, rheumatism, and endocarditis with dyspnoeic symptoms. The total estimations reported number over 170.

While increased alkalinity is not peculiar alone to sera from cases of malignancy, it is considered noteworthy that this should be such a constant feature of this pathological condition.

### 7. Changes in the Alkalinity of the Blood Following the Experimental Removal of Organs

*Dr. Maud L. Menten* (St. Louis): The immediate effect of the removal of internal organs was an increased acidity of varying duration. Curves were shown indicating the part played by anesthetics and by shock in the production of this acidity. The subsequent effect obtained depended on the organ removed. After total extirpation of the adrenals or the liver, increased acidity was found; following complete removal of the stomach, pancreas, or spleen, the serum became more alkaline. A similar condition resulted after pan-hysterectomy.

The normal serum reaction was maintained when only partial removal of these organs was performed.

#### DISCUSSION

*Dr. E. T. Bell* (Minneapolis): I should like to ask Dr. Menten if the functional condition of the kidneys was controlled in these cases of carcinoma by the phenolsulphonephthalein test?

*Dr. Menten:* No; the ordinary urine examinations were made, however, most of which I did myself. I should say that in about 80 per cent of the cases there was no albumin and a normal amount of urine was passed.

*Dr. Wells:* I should like to ask whether those cases of diabetes accompanied by cancer showed an increased alkalinity?

*Dr. Menten:* In those cases there was a high alkalinity.

## 8. The Influence of Certain Dietary Factors upon the Growth of Experimental Tumors

*Dr. Stanley R. Benedict and Mr. Alfred H. Rahe* (From the Huntington Fund for Cancer Research, Cornell University Medical College, New York City): The work was planned to study the influence of the accessory substance or substances in food which are necessary for body growth, upon the growth of the Buffalo sarcoma in white rats. Forty young white rats were employed, having an initial weight of from 30 to 60 gm. These animals received the Funk-Macallum diet, and in addition a few milligrams of dried brewers' yeast mixed with butter was given to each animal every day. On this diet the rats did not grow perceptibly in a period of three weeks. At the end of this time the animals were all planted with the same Buffalo sarcoma, and then divided into two equal lots. One of these (lot A) continued to receive the diet deficient in growth-promoting substance, while the second group (lot B) was given a diet of bread and vegetables, upon which body growth took place normally. The results showed that the number of takes was the same in each lot. In lot B, however, the rate of growth was many times faster than in lot A. The total weight of rat plus tumor in the animals upon the deficient diet did not increase appreciably. From these results we conclude tentatively that tumor cells, like those of somatic tissue, lack the power to grow in the absence of certain diet accessories.

The work is being continued.

### DISCUSSION

*Dr. Isaac Levin* (New York): Dr. Benedict's paper is of great theoretical interest, as the demonstration of a relation between nutrition and tumor growth might, perhaps, help to lucidate the genesis of cancer. I have recently conducted a similar investigation with the Rous chicken sarcoma at the Crocker Laboratory, in the course of which several hundred fowls were used. The chickens were fed only with highly polished rice, and as a result of the lack of vitamin in their food became extremely emaciated, while a certain number developed polyneuritis as a result of the faulty diet. Notwithstanding all this, the sarcoma grew as rapidly in these chickens as in the properly fed controls.

*Dr. Benedict* (closing): The experiments of Dr. Funk with the Rous sarcoma were not very conclusive either way. The objection to taking those experiments as at all conclusive is that we lack proof that the growth vitamin is the same thing as the beri-beri vitamin. The fact that fowls develop a polyneuritis after being fed with polished rice is no proof that the growth factor is involved at all.

## 9. Report of a Case of Multiple Tumor-Formation; Pathologic Changes and High Cholesterin Value in the Blood

*Dr. Georgine Luden*, (Rochester, Minn.): The occurrence of multiple tumors in a number of patients, and of malignant proliferation in no less than four different tissues in one patient, seems to indicate the presence of some fundamental factor capable of causing a malignant reaction in any part of the body when associated with traumatic lesions, even though the latter in themselves be insignificant.

A microchemical reaction (affinity for basic dyes) found in malignant areas suggests that this fundamental factor stimulating cell proliferation may be of a chemical nature.

Experimental observations show that the rate of cell division is increased by the presence of cholesterin, a substance that is definitely chemical.

High cholesterin values have been found in the blood of patients suffering from malignant disease, and an unusually high cholesterin value could be demonstrated in the blood of a patient in whose tissues a generalized tendency to malignant proliferation was also demonstrable.

The pathologic changes found in the adrenals in the patient under discussion were comparable to those found in the adrenals of animals subjected to extreme cholesterin feeding.

Metabolic disturbances preceded and accompanied the progress of the disease, and seem to warrant the assumption that faulty metabolism resulting in an accumulation of cholesterin is closely connected with cell proliferation.

It has been proved that the liver plays an important part in the regulation of cholesterin metabolism. Unmistakable evidence of malignant proliferation was found in the liver of the patient in whose blood the highest cholesterin value had been observed. Consequently,the deduction seems admissible that the integrity of the liver function plays an important, perhaps a prominent, part in the etiology of malignant disease.

This deduction appears to be corroborated by clinical and experimental evidence concerning the influence of heredity on malignant conditions, an influence that has been generally conceded and may be readily explained as due to the transmission of inadequate organs.

### DISCUSSION

*Dr. E. R. LeCount* (Chicago): In the post-mortem examination of human bodies there are not infrequently discovered in the liver and the kidneys, sharply outlined, yellowish areas a millimeter or less in diameter, often quite vivid against a background reddish because of the blood content. I have examined a large number of these, and have found them to be fat and doubly refractive, probably cholesterol. Dr. Katherine Dewey who has now in press an article[1] dealing with experi-

[1] Arch. of Int. Med., 1916.

mental cholesterolaemia, found similar areas in the kidneys of animals after cholesterol had been introduced directly into the blood stream; this condition she likens to the "large white kidney," in a measure. One interesting result of her work was the discovery of cholesterol as small gall stones in the gall-bladders of rabbits thus injected with cholesterol.

*Dr. Wm. B. Coley* (New York): I think Dr. Luden's case, as reported, is in harmony with clinical observations, and I believe that the occurrence of various types of tumor in the same individual is more common than is generally supposed. Indeed, I can recall five such cases which have come under my own observation, in one of which two types of tumor were present, while in two others there were three varieties. One of these cases, which I reported before the American Surgical Association in Washington three years ago, was a periosteal sarcoma involving two-thirds of the shaft of the femur; this tumor Dr. E. K. Dunham pronounced a small round-cell sarcoma. The patient had x-ray treatment for several months, in 1902, with temporary diminution in the size of the growth. A few months later, extensive metastases developed in the pectoral region and the ilium, which disappeared under the mixed toxins of erysipelas and bacillus prodigiosus. Ten years later, probably as the result of long continued irritation from the x-ray treatment, which caused a severe dermatitis of the thigh, a tumor developed at this site and grew with great rapidity. A specimen was removed, and pronounced sarcoma by Dr. Ewing, and carcinoma by Dr. W. C. Clark. Another specimen was examined by Dr. Wm. H. Welch, who found carcinoma in one portion, and sarcoma in another. Shortly afterward I amputated the thigh, and found a tumor, a typical epithelioma, about the size of an English walnut, occupying the medullary portion of the femur. The external neoplasm, which had started in the region of the old x-ray dermatitis, had no connection with the bone. Part of this growth proved to be large round-celled sarcoma, while other parts were definitely carcinoma.

Another case I reported before the New York Surgical Society in October, 1897. The patient, a female, fifty-three years of age, was operated upon in the spring of 1894 for a carcinoma of the left breast involving the axillary glands. She remained well until the fall of 1895; she then noticed enlargement of the maxillary glands, which continued to increase in size until the beginning of 1896, when they had produced a tumor 7 cm. in diameter, firmly fixed, beneath the angle of the jaw on the right side. The patient was put upon the mixed toxins and after four weeks' treatment the nodule had decreased two-thirds in size and was very movable. On March 14, 1896, I operated, removing two growths, one nearly 2 and the other $2\frac{1}{2}$ cm. in diameter, and situated in the deep submaxillary region; both were entirely encapsulated and both, macroscopically, were apparently sarcomatous. Drs. E. K. Dunham and B. H. Buxton examined specimens at the Memorial Hospital, and stated that there was no evidence of malignancy, but that they re-

garded them as glandular hyperplasia. In January, 1896, a recurrence of the original breast tumor (removed in 1894) having taken place in the pectoral region and axilla, involving the skin and subcutaneous tissue, I removed the entire diseased area together with both pectoral muscles. Microscopical examination showed the growth to be a typical scirrhous carcinoma. Shortly after this a rapidly growing recurrence of the cervical tumor was observed, and in September, 1896, I again operated, removing an encapsulated tumor the size of a small egg. This was diagnosed by Drs. Dunham and Buxton as small round-celled sarcoma. About a month later, a second recurrence took place in the submaxillary region, and grew with great rapidity; the tumor in the neck had the soft, cystic appearance of a rapidly growing round-celled sarcoma. The disease progressed forming a huge tumor of the neck and mouth which caused extreme difficulty in speaking and swallowing. The patient finally died of exhaustion on March 18, 1897, at which time there was a slight local recurrence of the carcinoma in the breast. Repeated examinations of the tumors removed from the neck showed no trace whatever of epithelial proliferation. There seemed little doubt that we were dealing with two different types of malignant disease in the same individual.

My third case was a carcinoma of the breast, which developed nearly ten years after an intra-abdominal sarcoma had been removed by Dr. McBurney, and in which the pathological report was confirmed by the records of the Roosevelt Hospital.

The fourth was a tumor originating in the region of the anus, which was pronounced a primary sarcoma by Dr. Maurice Richardson of Boston. The recurrence which developed later proved to be a typical carcinoma. In this case I had no specimen of the primary tumor to compare with the secondary, and I think there may be room for a little doubt.

The fifth and most recently observed case, is still in the hospital. This patient had a spindle-celled sarcoma of the uterus six years ago which was regarded as inoperable, after an exploratory operation by a Boston surgeon, and she was referred to me for toxin treatment. After six months' treatment the tumor became much more movable and, I believed, operable; in January, 1912, therefore, with the assistance of Dr. Downes, I performed a hysterectomy, removing a tumor somewhat larger than a child's head. This was pronounced spindle-celled sarcoma by Dr. W. C. Clark. The patient remained in good health until a few months ago, when she developed a tumor in each breast. These growths, which appeared to be benign, were regarded at that time as adenomas. The one from the left breast proved to be an adenoma, but the one from the right showed cells which, Dr. Ewing believed, were suggestive of carcinoma. A few weeks ago there was evidence of a local return, and I immediately removed the whole breast and the axillary nodes; both were examined by Dr. Ewing, who reported typical carcinoma.

In regard to the cholesterin, I should like to ask if it is possible that

the cholesterin content may be the effect rather than the cause, and if we may not as well assume a specific irritation; for example, some microorganism which, in some cases, stimulates one type of cell, in other cases, another type, both varieties of the organism occurring in the same individual in these rare instances of two or more tumors. That idea may perhaps be supported by recent experiments of A. S. Leyton, who obtained a pure culture of the streptothrix from rat sarcoma with which he was able to reproduce the disease repeatedly in other animals. He also found that the streptothrix developed spores which would pass through a Chamberland filter, and that with this filtrate he could produce the disease.

*Dr. Richard Weil* (New York): I think it is evident to all of us that the programs of the Cancer Research Society are changing to a certain extent, and that we are getting a great many more papers on the metabolism of tumors. These not only discuss the effects of alteration in diet, as with Dr. Benedict's experiments, but in some cases try to throw light on the cause of cancer, and it seems to me that we ought to be very careful before we accept the conclusions of the latter type of experiment.

Dr. Coley has described a number of instances in which he observed multiple primary malignant growths. If one looks up the data in Wolff one finds that this condition is very rare; malignant tumors do not tend to occur twice in the same individual. The few cases that are found might be explained by coincidence, since it would be rather striking if there were not a few cases in which different organs in the same individual were affected. But I think it is going far afield to suppose that these individuals must have some special predisposition toward malignant growth.

As regards Dr. Luden's individual case, I should not like to offer any theory, but I do not believe that her contentions have been clearly proved. It seems to me that until we have experimental evidence that certain alterations of diet or of metabolism will result in a change in the incidence of tumors, we ought to be just as chary of accepting such an explanation as we have been in the past of accepting the microbic theory.

*Dr. Luden* (closing): I do not maintain that the percentage of cholesterin found in the blood by any means settles the cancer question, but it strikes me as worthy of note that just as an increase of cholesterin has been found in the blood of a number of cancer patients, so an increase has been found during normal pregnancy; but in the latter case the percentage descends to normal after labor, that is after the child is no longer dependent on the mother organism for its growth, while in cancer there is a lawless and generally continuous growth. From this point of view the experiment of Burnett and Robertson seems specially interesting. Burnett and Robertson divided 12 rats bearing tumors of the same size into two groups of 6 each, and injected one of these groups

with cholesterol solution (intravenously); in a given time the tumors in these rats were twice the size of those in the other six. In consideration of these facts, the extremely high cholesterin value found in the blood of a patient with multiple tumors seems to me specially significant.

## 10. The Occurrence of Structures Resembling Embryomata in Crown Gall

*Dr. Erwin F. Smith* (Washington): Dr. Smith stated that in the time allotted to him he could not do more than show a few slides. He believed he now had evidence that the skin and glands of plants can be made to proliferate in a cancerous manner by bacterial inoculations but he would at this time confine his remarks to embryomas, of which he had produced a large number during the last four months. He then spoke substantially as he has written in the April number of this Journal, but exhibited additional photographs and photomicrographs of bacterially produced embryomas, laying special stress on those brought about in tobacco leaves where no totipotent cells are known to exist. Some of the more striking of the embryomas obtained in leaves have been figured in *The Journal of Agricultural Research*, 1916, April 24. Many of the lantern slides exhibited were in colors (Lumière and Hess-Ives processes). Several were shown illustrating malignant enlargement of glands of Ricinus in the vicinity of tumors.

### DISCUSSION

*Dr. James Ewing* (New York): I am sure that Dr. Roger Williams would be delighted to hear of the experimental production of teratomas in plants. Williams has long committed himself to the view that tumors arise by budding. In his book on the natural history of tumors he has an illustration of teratomas in plants. Probably he had no idea that they could be produced experimentally on so large a scale as Dr. Smith has done.

There are different types of teratoid tumors. I have not had time to read Dr. Smith's communication in detail. I should like to know if single cells are involved in the production of these teratoids, or whether they involve several layers. In the first instance his tumors would accord with the rare group in the human subject where single cells are the source of the tumor, as seen chiefly in the sex glands. In the latter group he would deal with tumors corresponding with the embryonal type of local dermoids or teratoids. It would appear that these plant tumors correspond to the embryonal types of local teratoid tumors in the human subject. It would be interesting to search out the parallels between these tumors and those occurring in human beings, because they are a very complex group. It may also be of importance to ascertain what constitutes an inflammatory reaction to bacteria injected into plant tissue, if these teratoid tumors represent true neoplasms.

I am, of course, greatly impressed by the irritation origin of these

teratomas, and naturally look about for corresponding facts in the origin of human teratoids. As far as I know, there is no clinical information which would lead one to believe that human teratoids arise from any comparable cause as in the case of these plants. We have no evidence that the teratomas in man are of irritative origin, as are those Dr. Smith has produced. In view of his demonstration, I think pathologists and clinicians may turn their attention perhaps to the possibility that some such factors do enter into the origin of human mixed tumors.

*Dr. Leo Loeb* (St. Louis): I wish to point out one difference between the crown gall and the common galls in plants which resemble tumors. In the latter, a chemical stimulus (perhaps in combination with a physical one) causes a tumor-like proliferation of various tissues or the formation of typical organs at atypical places; these stimuli may be distance actions. In a similar way in Haberlandt's experiments the chemical and mechanical stimulus caused cell proliferation. But in these cases growth comes to a standstill after a period of activity, because the stimulus ceases to act. In the case of crown gall, however, the stimulus transmitted through bacteria propagates with the bacteria, and thus a continuously growing tumor is produced that more closely resembles animal cancer in this respect than do the other galls.

*Dr. H. G. Wells* (Chicago): I should like to mention the fact that in the literature of mouse tumors we see little or no mention of embryomata. This has impressed me as being rather strange, because they are far from rare in man. Miss Slye has found only one in over twelve thousand mice examined, in spite of the fact that we frequently get tumors of the testicle and ovary resembling the other tumors of these organs common in man. It may be that some other strain carrying mouse tumors might show a higher percentage of this particular type.

*Dr. H. R. Gaylord* (Buffalo): I should like to ask Dr. Smith how marked is the specificity of this organism in plants. I remember his first presentation of this subject, and from these varying pictures and his subsequent additions to the question it appears as though this organism were capable of producing tumors in an increasing variety of different plants. I should like him to state how specific and how limited it is in its activity on certain types of plants.

*Dr. Smith* (closing): In reply to Dr. Ewing's inquiry, I will say these tumors seem to me to be like the embryonal teratoids. I do not know that they are ever produced from a single cell. The teratoid parts are stimulated into development by the blastomatous (cancerous) parts, and they seem to me to be derived from groups of totipotent or nearly totipotent cells dislocated and stimulated by the growth of the blastomatous cells, rather than from a single totipotent cell.

In reply to Dr. Gaylord's inquiry, I would say that you cannot graft this tumor widely, but you can inoculate it widely by means of the

bacteria which are its cause. We have inoculated it, I should say, into more than thirty kinds of plants belonging to different families, but only very narrowly can the tumor itself be grafted. I should think that the results of inoculation in mice might, perhaps, depend upon the location of the graft; this, at all events, is true of plants. Had I inoculated in earlier experiments at the sites chosen this winter, I should have obtained these teratoids long ago. When the bacterially infected needle is thrust into the internode of a plant, a sarcoma follows; when it enters the vicinity of root anlage, a tumor bearing roots is the outcome. Inoculation at the site of dormant buds produces tumors covered with leafy shoots or flower shoots, and the interior of these tumors is as strange as their surface; i.e., they contain, beside blastomatous elements, fragments of young tissues of various organs variously oriented and fused, and often upside down. These fragments are bounded by membranes which normally would be on surfaces, and some of them bear glandular hairs.

## 11. Precancerous Dermatoses

*Dr. Walter J. Heimann* (of New York): About twenty years ago the relationship between certain dermatoses and their subsequent evolution into epithelioma was emphasized by Dubreuilh. Four years ago Bowen reported two cases exhibiting a fairly definite clinical picture which he regarded as precancerous, although they lacked evidence of malignancy; last year Darier added three, and Bowen one more. Darier termed the condition "Bowen's precancerous dermatosis." Three of the six cases reported were frankly malignant. Heimann saw another case which conformed clinically to Bowen's description, but lacked all evidence of cancer. All the cases mentioned were characterized by the presence of abnormal malpighian cells which were enlarged and vacuolated, and corresponded to structures seen in Darier's disease and Paget's disease.

There are numerous skin diseases now commonly classed as precancerous, the most important of which are naevi, senile keratomas, xeroderma pigmentosum, Paget's disease, Roentgen ray dermatitis, and scars (notably those due to burns, lupus, etc.). In comparison with the frequency of these supposedly precancerous conditions, however, their actual termination in malignancy is rare. Paget's disease is always related to mammary carcinoma, but whether the dermatosis is precancerous or postcancerous has not been determined. Xeroderma pigmentosum is perhaps the one truly precancerous dermatosis, but only as regards the disease taken as a whole, not as regards any given lesion, for it is impossible to foretell by any clinical or microscopic criteria which of the numerous lesions are predestined to malignancy. This latter fact, too, is applicable to all other dermatoses regarded as precancerous.

In a given epithelioma it is obvious that a precancerous stage must have existed, though this is unrecognizable by present methods. Nor do we know how often epithelioma arises from clinically normal skin, as

compared to the frequency with which it originates in these alleged precancerous lesions; such evidence as exists appears to indicate that the former far exceed the latter. Furthermore, the number of occasions on which such lesions do not become malignant greatly exceeds the number on which they do. It may be circumspect to allow such dermatoses to arouse suspicion of a cancerous outcome, and to place the observer on his guard; this, however, is a matter of individual experience, and is entirely different from a positive prophecy of malignancy. Therefore, as a clinical concept the term precancerous is a misnomer.

DISCUSSION

*Dr. Isaac Levin* (New York): If I understand Dr. Heimann correctly, his ultimate conclusion is that we can hardly assume the existence of a precancerous condition. The question of the existence of such a lesion is one of the most important chapters in the etiology of cancer, but I believe that investigations upon the skin have very frequently led us astray in cancer research. Indeed, it has always been my feeling that anything could be proved or disproved when epithelium is employed for the investigation. Thus, twenty years or so ago, when the question of parasitology and cell inclusions was the most prominent aspect of cancer research, the discovery of a new inclusion or of a new parasite could always be confirmed on an epithelioma. Hence it is my conviction that gastric ulcer and other similar lesions are more suitable for the investigation of this subject. Four years ago I reported an experimental study in which the presence of a precancerous condition seemed to be quite clear. A carcinoma of the white rat which did not grow when inoculated in the normal testicle grew well in the testicle when this organ had been previously treated by substances which produced an inflammatory connective tissue proliferation. This research presents a clear-cut experimental proof of the existence of a precancerous condition. In the human subject, however, it is a great deal more difficult to decide which phenomenon is the cause and which is the effect. Dr. Heimann said that the transformation of lupus vulgaris into cancer is rare. This is due in many cases, I believe, to the youth of the patient. In regions where their life is prolonged, epithelioma is found more frequently; thus in Hamburg, Germany, I have seen a number of epitheliomas which had developed from lupus vulgaris.

*Dr. James Ewing* (New York): Dr. Heimann reports that in a certain group of epitheliomas he found so many which were, and so many which were not preceded by precancerous changes. I should like to inquire how he eliminated the existence of precancerous changes in those which were not so preceded.

*Dr. Heimann* (closing): As regards Dr. Levin's remarks, I find but one point to discuss in them. The infrequency of lupus vulgaris as a predisposant to cancer I have observed by studying a large number of

cases of lupus in European institutions; although lupus begins in young individuals, the disease persists throughout life without tending to shorten it, unless complicated by pulmonary or other forms of tuberculosis; nevertheless, it is rare to see it end in epithelioma. I have seen epitheliomas (I do not know how many) but I cannot think of more than three or four in connection with lupus. Hence, I do not believe that this disease has a great deal of bearing on this question—any more, in fact, than has been indicated in the paper.

As regards Dr. Ewing's question, I said that there was no clinically ascertainable precancerous dermatosis in these cases, but I assumed that there was a precancerous stage. What I object to is the designation of a lesion as precancerous when fifteen or twenty similar lesions on the body never become cancerous. Of course, if the patient had lived longer some of these might have become cancerous, but that is prophecy. I assume, as I said before, that there must be a precancerous stage, but I do not see how this can be recognized clinically.

## 12. Gastric Tubules in Early Gastric Cancer

*Dr. Louis B. Wilson* (Rochester, Minn.) (see this issue, p. 357).

## 13. Metastasis of Cancer in the Central Nervous System[1] (An Experimental and Clinical Study)

*Dr. Isaac Levin* (New York): Statistical investigations show that metastases in the brain are present in only 4.77 per cent of all the autopsies on cases of carcinoma in which the brain is examined. In view of the fact that metastases in other organs are present in more than 50 per cent of cases of carcinoma, metastases in the brain must be considered rare.

The experimental investigation of the writer confirms the opinion of those authors who maintain that the infrequency of metastases in the central nervous system is not due to the mechanical difficulties of transportation of cancer emboli, but to an inhibitory influence exerted by the tissue of the brain upon the development and proliferation of cancer emboli lodged therein. The experiments consisted in the inoculation of the Rous chicken sarcoma into the various organs of the animals, the creation, in other words, of artificial metastases. When the tumor was inoculated into the pectoral muscle, the liver, or the gizzard, there developed a large tumor, which produced metastases in other organs. On the other hand, when the tumor was inoculated into the brain there arose only small growths. Moreover, among 57 animals in which the tumor was inoculated into the brain, in not a single instance did a metastasis form anywhere. When a tumor was inoculated from the brain into the pectoral muscle of another chicken, it did not develop

[1] The original paper will appear in this Journal.

into a large tumor, and only after two subsequent reinoculations did the tumor nearly regain its original virulence. All this tends to show that the malignant characteristics of the sarcoma of the fowl are impaired by sojourn in the brain.

The clinical investigations of the writer also seem to bear out the opinion that metastasis in the brain is rare. During the last three years he has observed 3 cases of metastasis in the brain, of which one came to autopsy. The peculiarity of all three consists in the fact that they pursued a very malignant clinical course, and that the metastases in the brain appeared comparatively late in the course of the disease. The impression may be gained from an analysis of these three cases, that the brain resists the invasion of carcinoma longer than the other organs. Metastases in the meninges are still less frequent, and only 7 cases are mentioned in the literature with a metastasis of carcinoma in the cord. On the other hand, metastases in the vertebrae are more frequent and are usually followed by symptoms indicating disease of the cord; the writer has recently had the opportunity to study 3 such cases. In these cases, the primary tumor frequently causes no symptoms and therefore the correct diagnosis is not made, unless the clinician keeps in mind the possibility of malignancy and examines the spine radiographically as a routine procedure in all obscure cases of spinal disease.

DISCUSSION

*Dr. H. G. Wells* (Chicago): Our examinations of the brains of mice were not made with particular reference to metastasis, but chiefly in cases where we could not determine the cause of death. It will be recalled that all our mice were autopsied, irrespective of the cause of death. As the material is still on hand the brains can yet be examined, and I think it would be very interesting to see how often they contain metastases.

*Dr. H. R. Gaylord* (Buffalo): Dr. Levin has emphasized the fact that the Rous sarcoma, when inoculated into the brains of chickens, kills while the tumor is still very small. I should like to ask him if he was able to explain this early death on anatomical grounds, or whether generalization of the virus occurs earlier in the case of cranial tumors than it does when the neoplasm is situated in the muscular structure of the breast. Dr. Levin probably remembers that Dr. Clowes and Dr. Simpson last year succeeded in finding the virus in the blood of moribund chickens by citration and filtration.

*Dr. Levin* (closing): I shall be glad to hear the result of Dr. Wells' examination of the brains of his tumor mice. The reply to Dr. Gaylord's question, I believe, could be only hypothetical. I do not know the exact cause of death in these chickens, as I was mainly interested in the fact that the animals lived about 12 days; consequently it was possible to compare the results with those in which the tumor was inoculated into other organs.

## 14. The Effect of Certain Anesthetics and Loss of Blood upon the Growth of Transplanted Mouse Cancer

*Dr. H. R. Gaylord and Dr. Burton T. Simpson* (State Institute for the Study of Malignant Disease, Buffalo, N. Y.): The authors referred to experiments prosecuted at the State Institute in 1905 in which it was shown that stationary tumors (transplanted mammary carcinoma in mice) could be caused to grow rapidly by bleeding, either one large hemorrhage or repeated small losses of blood producing this effect. This was supplemented by experiments showing that chloroform and ether, used as anesthetics, could in many instances cause stationary tumors to grow rapidly. These facts are apparently confirmed by the careful studies of Tyzzer with the Japanese waltzing mouse tumor, in which he showed that the removal of primary tumors caused metastases to grow to a larger size in a given time than was the case in control animals where the primary tumors were not touched. Tyzzer also showed that secondary implants and recurrences were influenced by operation. The speaker has been informed by Dr. Tyzzer that he employed ether in his operations.

For the purpose of testing again the question of hemorrhage and anesthesia upon the growth of transplanted mouse cancers, experiments were planned with a propagable mammary carcinoma which is giving a very consistent inoculation outcome of 50 per cent. The animals used in the experiments were comparable as to size, age, and sex (males). They were divided into three lots of 20 each, the first of which was inoculated with small fragments of tumor by the trocar method. Immediately after inoculation 10 of the mice, Lot 1801, were anesthetized with chloroform, which was done by placing them individually in a glass jar and spraying enough chloroform with an atomizer to cause them to fall over. This short anesthesia was repeated each day until, on the tenth day, 9 out of 10 (90 per cent) of the anesthetized mice had developed palpable tumors of considerable size. In the control series of 10, Lot 2804, 5 out of 10 (50 per cent) had tumors. In the anesthetized animals, the tumors grew very rapidly and there were no spontaneous recoveries, while among the controls, 2 of the 5 animals which took tumors recovered spontaneously. Two lots of 10 mice each were used in a similar way, with ether as the anesthetic. 10 mice inoculated by the trocar method with small fragments of tumor were anesthetized each day with ether, Lot 2692, the other lot being used as control. On the twelfth day, one mouse had died, and of the remaining 9, 6 had developed tumors ($66\frac{2}{3}$ per cent), whereas, 5 (50 per cent) of the controls, Lot 2694, had growths. In the anesthetized series there was one spontaneous recovery, and one also in the control series.

To test the effect of loss of blood, 10 mice, Lot 2691, with their controls, Lot 2693, were inoculated as in the preceding experiments, and in Lot 2691 approximately 3 drops of blood were allowed to flow from the tail vein each day, the wound being cauterized to prevent infection. On the twelfth day, each lot was examined. In the mice which had

been bled, Lot 2691, 8 of the 10 mice (80 per cent) showed tumors, and of the controls, 5 in 10 (50 per cent). There was one spontaneous recovery in each lot.

From the experiments here recorded it would appear that chloroform exerts a pronounced effect upon the natural resistance of mice to the transplantation of this tumor, and that ether has a less definite influence. The daily loss of blood has an effect almost equal to that of chloroform. It would appear that the effect of hemorrhage and of anesthesia accounts in large part for the increased rate of growth noted in recurrent cancer, secondary grafts, and metastases after operation; that loss of blood and the use of anesthetics appear to injure the natural resistance of a certain number of mice.

Repeated loss of blood or anesthesia with ether, when begun on the day of inoculation and continued until the appearance of the tumor and then discontinued, may be followed later in individual instances by spontaneous recovery. As the earlier experiments indicated that loss of blood or anesthesia inaugurated a period of rapid growth, it will remain to test the question whether hemorrhage and anesthesia are capable of overcoming the resistance following spontaneous recovery. Such experiments are now under way, and are sufficiently advanced to indicate that animals rendered resistant by spontaneous recovery, or by repeated unsuccessful inoculations, cannot be rendered susceptible in the manner in which these experiments have been carried out. Fourteen spontaneously recovered mice and fourteen mice immune by reason of unsuccessful inoculation, were reinoculated, and both bled and chloroformed each day until the control tumors were well established. In no instance did one of these mice develop a tumor. Experiments in which the bleeding and anesthesia preceded inoculation are now being carried out, and the relation of the immunity induced by spontaneous recovery or by unsuccessful inoculation, to anesthesia and hemorrhage, will form the subject of a subsequent paper.

## DISCUSSION

*Dr. E. E. Tyzzer* (Boston): In my investigation of this question of the growth of metastases after operation, the hypothesis of Dr. Gaylord was not considered. I found that after operation the metastases grew larger in a given period of time than they did in control animals; that is, the rate of growth in the metastases was more rapid. My hypothesis was, that the removal of the primary tumor made so much more nutritive material available for the growth of metastases; but it must be admitted that Dr. Gaylord's suggestion should be taken into account. I should like to see the experiment done, however, on a group of homogeneous animals. In mice which produce only 50 per cent of tumors, I should expect more or less variation, different groups having different inoculation percentages. Therefore it is necessary to use a rather large number of animals before drawing conclusions.

*Dr. Isaac Levin* (New York): I should like to know for how long a period of time the animals were kept under the anesthetics; and I do not think the speaker mentioned what tumors were employed. Different mouse tumors behave in different manners. While I have no direct experience in bleeding animals, I have had extensive experience in anesthetizing them, having done a large number of inoculations into parenchymatous organs under general anesthesia; and I have usually found the percentage of takes in the parenchymatous organs similar to that following subcutaneous inoculation. Consequently, in my experiments at least, the anesthesia did not appear to exert any influence on the growth of the tumor.

*Dr. H. G. Wells* (Chicago): In connection with the explanation that this difference possibly depends upon immunity, I would call attention to observations made by Dr. Hektoen, that repeated bleeding, or sometimes a single hemorrhage, is commonly followed by increased production of antibodies, the explanation being that the bleeding stimulates the hematogenic function and consequently the production of antibodies. The results of anesthetics upon antibody formation, however, are exactly opposite to those of bleeding, and hence are not harmonious with the conclusions drawn from these experiments.

*Dr. Gaylord* (closing): I must admit Dr. Tyzzer's criticism about non-comparable material. In my manuscript I have pointed out that only specially bred and selected mice could be called comparable, and even then we are far removed from strictly comparable material, even when the mice are of the same parentage. I would lay particular stress upon the fact that for two years this particular carcinoma has never given over 65 per cent of positive inoculations, but has maintained a constant average of 50 per cent, falling occasionally to 45 per cent and rising once in a while to 55 per cent; 65 per cent, however, is the exception, and a 90 per cent inoculation result, as in the case of the experiments with chloroform, could, therefore, scarcely be more striking. The figures relating to ether are not entirely convincing, but viewed in the light of the results with chloroform are at least suggestive; 80 per cent in the bleeding experiments is certainly a very distinct rise. In the light of Dr. Tyzzer's carefully planned experiments, as well as our own early observations, the figures appear very forceful, and in reading his paper it seemed to me that the greater part of the effect produced by surgical operation might be attributable to the loss of blood and anesthetics.

Dr. Levin has remarked that in operations upon the chicken sarcoma he invariably observed no such effect. In this connection it may be said that in our experience the chicken sarcoma has for a long time been very virulent, in many instances giving almost 100 per cent on transplantation. Under such conditions, of course, a greater susceptibility to inoculation would not be observable, and the effect, no doubt, would be found in very rapid growth which, if not directly sought for and properly controlled to determine its existence, would escape detection.

As to the manner in which chloroform and ether increase the rate of growth, I have no explanation to offer. Dr. Wells' remark that loss of blood tends to produce more antibodies in animals, and anesthetics to destroy antibodies, would suggest that hemorrhage and anesthesia do not bring about a destruction of resistance through the same mechanism. It is a question, however, which I should prefer to leave open. This particular series of experiments bears upon the phenomenon of natural resistance in mice, and, as applied, the experiments do not affect acquired immunity following spontaneous recovery. The essential fact is, that hemorrhage and anesthesia will cause existing tumors to grow more rapidly, and that they should therefore be of distinct significance to the surgeon.

## 15. Further Investigations on the Hereditary Transmission of Differences in Susceptibility to the Growth of Transplanted Tumors in Various Strains of Mice

*Dr. Moyer S. Fleisher and Dr. Leo Loeb:* It is generally admitted that the differences in susceptibility to the growth of a certain tumor in animals belonging to different species and varieties, are based on constitutional differences in the animals. Thus it is understood that a mouse tumor cannot grow for any length of time in a rat, and that a tumor found in a common white mouse will ordinarily not grow even in a wild grey mouse or in a waltzing mouse. Michaelis, Haaland, and others have shown, however, that even among animals of the same kind, different strains and families which structurally appear to be identical differ markedly as to their susceptibility to the growth of the same tumor. A number of investigators are inclined to attribute this latter difference not so much to constitutional differences, which are hereditarily transmitted in certain families, as to external conditions, and especially to the food. Hence they believe that this susceptibility can be changed experimentally.

Our investigations lead to the following conclusions:

1. Differences in susceptibility to the growth of inoculated tumors, observed in various strains of the same variety of animal, are due to inheritable constitutional causes, or, in other words, to a specific adaptation of tumor cells to the soil in which they originated; they are not due to the expression of temporary changes in environment, especially food. An apparent exception to this rule appeared in our experiments, namely, the breeding out of a pure line that differed in susceptibility from the rest of the strain.

2. In further experiments we could not obtain the same typical variations in the susceptibility to the growth of inoculated tumors of successive generations of hybrids which were formerly observed by Tyzzer and ourselves. The same typical variations were also absent in the successive generations of hybrids between wild mice and domesticated American mice.

## 16. The Inheritability of Spontaneous Tumors of the Liver in Mice

*Miss Maud Slye* (Chicago): During the past three years I have presented before this Society evidence showing the inheritability of spontaneous cancer in mice.

This report presents evidence of the persistence, in certain strains of mice, of spontaneous tumors of specific organs and of specific types, when determined both by inbreeding and by hybridization.

Such a study cannot be undertaken until a large number of tumors has arisen in a very extensive stock, for with the exception of mammary gland tumors and of lung tumors (which constitute about 90 per cent of all mouse tumors) the cases accumulate slowly.

I have selected, as the centre of this presentation, tumors of the liver, since they are sufficiently rare in the literature to form an interesting study, and sufficiently numerous in this stock to make a striking one. In all the literature there is, so far as I know, but one tumor of the liver reported, viz., an adenoma of the liver described from the Imperial Cancer Research Fund of London. The Slye stock has furnished to date 58 primary tumors of this organ.

This study is based on a living population of about 12,000, which for the past year has produced a steady output from cancer strains of from fifty to one hundred cases of cancer. There have been performed more than 13,000 autopsies, involving over 2000 primary spontaneous neoplasms.

Of these 2000, fifty-eight were primary in the liver; these were chiefly adenomas, though a few sarcomas and carcinomas were discovered among them.

In distribution they have fallen exclusively in those cancer-bearing strains into which they have been bred:

*Negative Evidence.* It is possible to show a very large number of strains of mice carrying from 50 to 100 per cent of cancer without the occurrence of a single tumor of the liver.

*Positive Evidence.* Strains of mice derived from ancestry with tumor of the liver nearly always show somewhere an outcropping of liver tumor. Frequently they show three or more successive generations with liver tumor, often with more than one case in a single generation.

When we consider that only one such case has been reported heretofore, this persistence of liver tumors in the strain into which they have been bred, is indeed striking.

Whatever the ultimate nature of cancer, and whatever the ultimate mechanism of heredity (both unknown) may prove to be, as this most prolific stock of cancer-bearing mice grows older and broader, it furnishes increasingly stronger and more complete evidence of the following: that not only cancer in general, but tumors of specific organs and of specific types, persist in strains where they have been bred in. And this evidence remains after every possible test has been carried on for years, in the effort to transmit cancer from one individual to another by some other mechanism than that of heredity.

DISCUSSION

*Dr. C. C. Little* (Boston): I think this is an extremely interesting set of charts, and that they represent a step along the line of progress. They show beyond reasonable doubt that there are different hereditary tendencies underlying different types of tumors. This is one of the chief points that I have tried to bring out in what discussion I have had with Miss Slye, and one which would strongly support and, in fact, seem to prove definitely, that cancer, in its inheritance, is *unlike* albinism. It is a very much more complex subject, and these charts showing tendencies in a given strain to one type of tumor and not to another, offer evidence which indicates that a point will be reached eventually where it will be possible to analyze the hereditary factors more fully.

In looking over the majority of the charts here it is striking to notice that a distinctly higher proportion of all the types of tumors recorded occurred in female mice; this appears to indicate that there is a particularly important line of reasoning to follow out, because cancer is a product of reactions of the internal environment, and one of the most important controlling factors in the internal environment is the secretion of the *sex glands*. Now Loeb's recent work on mice in which the ovaries had been extirpated has shown that removal of that particular factor has caused a disturbance in cancer incidence, and it seems to me that here again we see an indication of the complexity of the hereditary factors involved. Sex is undoubtedly alternative in its inheritance; we do not get blends of males and females. These charts appear to show strikingly the important part that sex plays in the incidence of spontaneous tumors. I do not mean to say that sex is the all-important thing, but that what we are really dealing with is a very complex reaction of the internal environment, and for that reason it is necessary to be extremely careful in comparing such a character, in its inheritance, with the simpler types. The general facts of the inheritance of cancer are amply shown by the work of Miss Slye, but to suggest in any way that it bears any relation to a simpler character would be erroneous.

*Dr. Isaac Levin* (New York): I am sure that all have followed with great interest Miss Slye's work on inheritance, and her investigations are certainly fruitful in interesting deductions. To me the most suggestive part of her results is the fact that there is apparently not only a general inheritance in spontaneous tumors, but also a specific inheritance for tumors of a certain kind, or of a certain organ. This observation appears to offer one more proof that cancer represents a complex group of various conditions, and can not, therefore, be caused by one infectious agent. Five years ago I investigated, by Mendelian methods, five families in which cancer was found to be somewhat more frequent than in other small New England towns. These five families included several thousand people, and one of the interesting points in the result of this work was that there also seemed to exist not only a general in-

heritance of cancer, but a specific inheritance of cancer of the same organ.

*Dr. James Ewing* (New York): In a previous discussion of Miss Slye's work before this Society I ventured to offer certain considerations which seemed to me of importance in their interpretation. One of them was that to group several different diseases as of equal significance in evidence of heredity was inadmissible. I especially objected to lymphosarcoma being included with fibro-sarcoma of the uterus, and urged that if we could get reports from Miss Slye on some particular form of tumor and its apparent relation to hereditary influence, the information would be more convincing. The other consideration that I mentioned, which came naturally from me as a pathologist, was that when we went out into clinical medicine and tried to see how far the influence of heredity applied, we did not get very impressive data; that, so far as the pathologist and the clinician could see, heredity did not play an important part in the incidence of most tumors.

Today Miss Slye has very successfully answered one of the criticisms, and presented a report of a single type of tumor in a way which must be very gratifying to her, as it is convincing to us.

In regard to the other objection, I must say that Miss Slye's work has pretty directly presented to the pathologist and the clinician the necessity for investigating cancer from the hereditary standpoint. She has engaged in this experimental work in the laboratory, and if the clinical evidence is not satisfactory it is not her responsibility to gather such evidence. This task belongs to the pathologist and the clinician. There are many human tumors in which the study of possible hereditary factors may prove fruitful. Cancer of the breast, which few regard as likely to be influenced by heredity, probably does not develop from the ordinary normal secreting parenchyma or ducts of the breast, but from redundant sweat glands not properly incorporated in the breast. My own material supports this view to a considerable extent. If such is the origin of cancer of the breast, the main factor is anatomical, and probably an embryonal disturbance of structure, which, of course, falls in the group of factors which are explained by congenital and hereditary influences. Pathologists and clinicians can profit by having this problem presented in such a definite manner as Miss Slye has done.

*Dr. E. T. Bell* (Minneapolis): I should like to ask Miss Slye what bearing her work would have on the classes of tumors that are almost altogether fatal before puberty, as for instance, the mixed tumors of the kidney in children.

*Dr. H. G. Wells* (Chicago): In regard to the question of the relation of sex, if Miss Slye has given the impression that there is a predominance of females over males it is because she has presented only part of her material. Our first twenty-eight cases of tumor of the liver showed

fourteen males and fourteen females, and I know that 160 cases of tumor of the lung were almost equally divided between males and females. As to the sarcomas upon which I reported this morning, when sarcomas of the mammary gland are omitted, they are almost equally divided between the two sexes; but the fact that a large proportion of sarcomas occur in the mammary gland makes the whole ratio two to one. So then, leaving out the question of the greater susceptibility of certain organs characteristic of one sex, it is found, after all, that there is not much difference between the two sexes. These findings are comparable with those encountered in human beings, where the preponderance of tumors in the female is due to those of the uterus and breast.

*Miss Slye* (closing): In replying to Dr. Little, I shall not at this time enter into any discussion of the comparison between albinism and cancer, a subject which is not germane to this paper. As I have previously stated in communications already published, any character which requires provocation, as cancer apparently does, is made complex by that fact. I should like, however, to re-emphasize the statement made by Dr. Wells. Dr. Little has gained an entirely erroneous impression if he thinks that four-fifths of the tumors of all types in this stock lie in the female. That is very far from the truth. When all strains have been tabulated, it is probable that there will be found some types of tumor which preponderate in the male; for example, the only two gastric tumors which have occurred in this stock, so far, were in males.

As for Dr. Ewing's suggestion, it was obviously impossible at the outset to make any study of the inheritance of tumors of specific organs and of specific types. It is, in fact, exceedingly difficult to carry a large number of cancer strains to anything like the age and numbers necessary to give an accurate test, even of the inheritability of cancer in general. As I said before this Society a year ago, it is impossible to study the inheritance of tumors of specific types and of specific organs until a very large number of neoplasms has arisen in an exceedingly large tumor stock; for with the exception of mammary and pulmonary neoplasms, the cases accumulate slowly. Thus, among these entire two thousand tumors there have been but two of the stomach, as has already been said, two of the uterus, a few of the kidney, etc. It was therefore impossible to draw any trustworthy conclusion on this point until last year, when I presented before this Society a paper on this subject covering carcinoma, sarcoma, tumors of the liver, ovary, mammary gland, lung, and other organs. It was useless to base such a study on mammary gland and lung tumors alone, since these constitute about 90 per cent of all mouse tumors, and might therefore be expected to predominate in any cancer strain.

Replying to Dr. Bell's question, so far as I can see his meaning I should say, although the facts have not yet been tabulated, that the occurrence of cancer in cancer strains is, on the whole, about as heavy in young mice born long before the parents have tumor as it is in young born after the occurrence of tumor in the parents—strong evidence of

the inheritability of cancer. For example, the parent female of this strain (No. 338) was mated with her brother long before she had tumor, and the young from that mating also showed tumor.

### 17. Studies on the Inheritance of Susceptibility to a Transplantable Sarcoma (J. w. B.) of the Japanese Waltzing Mouse

*Dr. E. E. Tyzzer and Dr. C. C. Little:* In the series of experiments here reported a sarcoma (J. w. B.) of the Japanese waltzing mouse was employed. The reaction of different classes of mice to this tumor was tested by the method followed in an earlier series of experiments[1] with a carcinoma (J. w. A.) which also originated in the waltzing mouse. The sarcoma grows upon inoculation in 100 per cent of Japanese waltzing mice. It also grows in all first generation hybrids obtained by cross-breeding this variety with common mice of two different races. It has shown continuous growth in two out of thirty-five common mice inoculated, and temporary growth in several others; while the carcinoma J. w. A. failed to show continuous growth in all of the ninety-nine common mice inoculated and showed temporary growth in only three. The sarcoma J. w. B. has grown in twenty-three of the eighty-nine $F_2$ generation hybrids, while the carcinoma grew in only three out of one hundred and eighty-three mice of this generation. A somewhat similar difference exists in the $F_3$ generation, where nine out of thirty-nine mice inoculated have grown the sarcoma and none of the thirty-eight inoculated grew the carcinoma.

The type of inheritance involved is clearly alternative, and the hypothesis offered in our previous paper to explain the transmission of susceptibility to the carcinoma will, with certain quantitative differences, apply to the sarcoma as well. The hypothesis is briefly as follows: Continued growth of either tumor depends upon the presence of a complex of independently inherited factors. This factor complex is present in a nearly homogeneous condition in animals of the Japanese waltzing race. Since $F_1$ hybrids have animals from the Japanese waltzing race as one of their parents, they will possess the factors comprising the Japanese complex in a "single dose." Since they grow the tumor it is evident that only a single representation of all these factors is necessary for susceptibility. On the supposition that the Japanese complex consists of many independent factors which segregate in a Mendelian fashion, we should expect that the exact combination of factors forming the Japanese complex would be seldom realized in animals of the $F_2$ generation. This would result in only a relatively small proportion of the $F_2$ generation growing the tumor continuously, the exact proportion being determined by the number of factors necessary for the

[1] Further experimental studies on the inheritance of susceptibility to a transplantable tumor, carcinoma (J. w. A) of the Japanese waltzing mouse—C. C. Little and E. E. Tyzzer. Jour. Med. Research, 1916, N. S., xxviii, 393.

growth of each tumor which, in the case of the carcinoma, is estimated at from twelve to fourteen and in the case of the sarcoma at from five to seven.

DISCUSSION

*Dr. Richard Weil* (New York): I should like to ask whether the tumor does not take at all in the common mouse, or whether it takes and then retrogresses.

*Dr. H. R. Gaylord* (Buffalo): I should like to ask Dr. Little if the two tumors he has studied, one being a carcinoma and the other a sarcoma, would not be expected, from their very nature, to show a greater specificity in the first instance than in the second. A carcinoma of the Japanese waltzing mouse might be considered a more highly specialized tumor than a sarcoma in the same strain. His figures, showing the almost complete specificity of the first and the less specific character in relation to transplantation of the second, would be in keeping with such an interpretation.

*Dr. H. G. Wells* (Chicago): I wish to ask one or two questions for information. I should like to know, first, what the histological structure of this sarcoma is, and whether it behaves differently from carcinomas; secondly, whether inoculation with carcinoma, for instance, prevents the sarcoma from growing, or conversely. The transplantation of mouse tumors has shown that the difficult distinction between sarcoma and granuloma is being correctly drawn, for tumors diagnosed as sarcoma and transplanted afford no higher inoculation percentage than do the carcinomas; and this, I take it, is very good evidence that granulomas are not being mistaken for sarcomas.

*Dr. E. E. Tyzzer* (Boston): When this tumor arose I had a great deal of difficulty in classifying it and am thus prepared for Dr. Wells' question concerning the nature of it. I was in doubt for a long time whether it was a carcinoma or a sarcoma. But the various differential stains give more evidence in favor of its being a sarcoma. There are fine collagen fibrils in close association with the tumor cells, it presents a perithelial arrangement, and there are fibroglia fibrils. Yet it is hard to say, after all, whether these fibrils belong to the tumor cells or to the cells of the host, and I admit that there is a great deal of difficulty in classifying such tumors.

I cannot quite understand the point of view which would question its neoplastic nature. This tumor has grown for many generations, just as transplanted carcinoma grows. Its great infectivity brought up the question whether it was not due to some organism, and on that account the tumor was ground up and filtered through two layers of filter paper. Now since this filtrate contained red blood corpuscles it was evident that particles as large as red blood corpuscles had passed through the filter paper, but no tumors have developed in the inoculated mice.

The inoculation of one strain of Japanese waltzing mouse tumor does not prevent the growth of the other.

*Dr. Little* (closing): Dr. Tyzzer, I think, has answered Dr. Wells' question about the histological structure of the sarcoma and about the inoculation of the tumor.

In regard to Dr. Weil's question, there has been a temporary growth for perhaps two or three weeks in three out of the ninety-nine common mice inoculated with the carcinoma mice and then a retrogression of the tumor. In the case of the sarcoma, temporary growth has occurred in several of the animals and been followed by a retrogression. All this, to my mind, suggests that even in the case of carcinoma certain of the common mice approach the constitution of Japanese mice without quite attaining it. There are fewer such mice in the case of carcinoma than sarcoma—again substantiating the higher ratios obtained in the $F_2$ and $F_3$ generations of animals inoculated with the sarcoma.

I believe that something is undoubtedly to be gained from a consideration of the relative specificity of these tumors. It was the very fact that we were beginning to find a difference in reaction of similar mice to the growth of different tumors, that encouraged me in believing that there is a different group of hereditary factors underlying their respective susceptibility.

## 18. A Study of the Serum Factor in Tumor Immunity

*Dr. Richard Weil* (New York): The cause of the acquired immunity of rats to implanted tumors has been the subject of considerable discussion, and is even now not satisfactorily determined. There are two main theories, one of which attributes the immunity to the presence of circulating antibodies, the other to cellular reaction. The latter will not be discussed in the present paper.

In 1905, it was maintained by workers in the Buffalo laboratory that the blood serum of mice which had recovered spontaneously from tumors possessed a power, when injected into mice with growing tumors, of inhibiting or causing retrogression of the latter. In 1913 I reported experiments of this type in rats, and found that it was impossible to affect the growth of tumors, whether prophylactic or curative effects were attempted. A recent paper by Dr. Tyzzer having emphasized again the arguments which theoretically, at least, indicate a participation of immune substances of the serum in tumor immunity, it seemed advisable to resume the study of this problem. The object of the present paper is chiefly to describe a new method which seems to offer distinct advantages over those previously employed in the examination of the serum for such substances. In the older methods, animals bearing tumors were injected with supposedly immune serum, or were injected with such serum previous to inoculation with fragments of tumor tissue. A possible objection to this technique is based upon the fact that the amount of tumor tissue to be acted upon is relatively large, and that

immune substances in the injected serum, even if present, might fail to reveal their activity when diluted by the whole volume of circulating blood of the injected animal. In order to obviate these difficulties, the following method was devised. The freshly removed sarcoma of the rat was finely minced with scissors and shaken up with salt solution. When the large particles had settled, the supernatant suspension of tumor cells was pipetted off; a part of this suspension was treated in given amounts with freshly drawn normal rat serum, another part with the serum of immune animals. A third part, untreated, was kept as a control. These mixtures were incubated for varying lengths of time, and were then injected in equivalent amounts into the jugular vein of a series of rats. A comparison of the pulmonary "takes" at the end of a period of two to three weeks permits of a definite conclusion as to the effect of the serum upon the tumor cells. The method appears to offer the advantage that the immune serum is brought into contact directly with the cells, that this contact is very much more intimate than is permitted by the older method, and that the period of incubation can be prolonged at will. If immune substances be present, it seems distinctly more probable that they would be detected by this method than by those hitherto employed.

Although a considerable series of rats has been injected, it is not possible at the present time to give a final report as to the results. Owing to the fact that the rats in the laboratory have been subject to an epidemic of broncho-pneumonia, the conclusions from many of the experiments have been impaired in value. From some of the data at present available, however, it seems probable that the immune serum in some instances affects the tumor cells in an unfavorable fashion. Thus in one series, the salt solution controls and the cells treated with normal serum took in 100 per cent of the rats, while the cells treated with immune serum took in only one-third.

As has been said, however, the data are still quite incomplete, and the final outcome of the series cannot be predicted at the present time with any certainty.

## DISCUSSION

*Dr. H. R. Gaylord* (Buffalo): Dr. Weil's experiments emphasize what has occurred before in the experience of investigators of the immunity to cancer. Not alone are his present positive findings of great importance, but the fact that three years ago, with somewhat differently planned experiments, he arrived at opposite conclusions, is illustrative of what has occurred in many laboratories.

Dr. Weil's suggestion that we did not hold the same opinion regarding our early experiments that we had in the beginning might cause a misunderstanding as to our position. We never departed from the theory that they were positive results and that as they stood they were significant, but attempts to extend them or to repeat them under other conditions were extremely disappointing. I set forth our posi-

tion in relation to passive immunity in an address before the Second International Cancer Congress in Paris in 1910, and pointed out then, as others have since, that attempts to apply our original observations to other tumors under other conditions had yielded very erratic results. We were, therefore, not surprised to find that Dr. Weil, a few years ago, was unable to demonstrate in the Buffalo rat sarcoma evidences of antibodies in the serum, although Lewin in Berlin had previously claimed positive results with a rat sarcoma.

As we look back on our earliest experiments we realize that the positive results then obtained were perhaps the maximum of expectation, and that the conditions were unusually favorable. In particular we feel that the fact that the Jensen mouse tumor at that time was declining in activity added to the striking character of the results. We were using the blood of animals recovered from the tumor in a more active stage to treat animals inoculated with the tumor at a less active stage. Even under these conditions the balance was very close and the evidence of immunity, although distinct, within narrow limitations.

Dr. Weil's emphasis on the exacting character of the experiments necessary to show the presence of immune forces in the serum in the Buffalo rat sarcoma, especially the use of finely divided suspension of cells, recalls Dr. Clowes' experiments in the test tube, in which he showed that a given amount of serum could only be made to affect a given number of cells.

Dr. Weil's paper is of the very greatest importance to the subject of immunity to cancer under experimental conditions, and that having once failed, he has again taken up the subject and pointed the way to further advances along this important line, is a matter for the heartiest congratulation.

*Dr. E. E. Tyzzer* (Boston): I think Dr. Weil is to be complimented on the very ingenious method which he has devised for this work, and I should be interested if he would some time follow the histology of these tumor emboli which he places in the circulation. I am convinced that there is some immune principle produced long before a tumor is destroyed, that evidence of this immune principle is to be found in the inflammatory reaction around the tumor, and that the tumor persists for some time after this reaction appears.

*Dr. James Ewing* (New York): Very convincing evidence of an immunizing action exerted by the serum was furnished by Beebe and Crile, who exsanguinated dogs bearing infectious lympho-sarcoma and then immediately transfused them with very large quantities of blood from immune dogs. In nine successive animals with very large tumors there was a complete regression of all the growths in every one. Whether that meant an active antagonistic factor in the serum or not, seemed to me uncertain. I was inclined to suspect, in a somewhat vague manner, that the serum furnished a more suitable nutriment for the body tissues, and that as a result of this increased nutrition, the tumors under-

went atrophy and disappeared. This idea has been very aptly expressed by Dr. Tyzzer, who suggested that the blood of the immune animal was more homogeneous with the normal tissues than was that of the infected animal.

*Dr. Gaylord* (Buffalo): Dr. Weil's advance in technique, the use of finely divided suspensions of cells which permits the demonstration of antibodies, is undoubtedly the method by which these subtle immune forces should be studied.

In this connection I am reminded of a very important observation made by Dr. Rous upon the chicken sarcoma, which he recently detailed to me in a verbal communication. Dr. Rous found that, whereas certain chickens were so resistant to the virus of spindle-cell sarcoma that repeated injections of this virus failed to develop a growth, he could still produce tumors by the implantation of grafts in spite of the immunity to the virus. This he interpreted as indicating that the virus of spindle cell sarcoma may resist the immune forces sufficiently to prevent a primary tumor through the protection afforded by the virus being within the cells at the time of implantation.

*Dr. Weil* (closing): As regards Dr. Ewing's comparison with the previous work on dogs, it seems to me that what he says is perfectly true. There are a number of factors, however, that made me feel that one ought to advance further along these lines. In the first place, there is still some doubt whether this disease of dogs is to be considered as a true tumor or as an infectious granuloma. In the second place, Beebe obtained, certainly more than once, retrogressions from normal blood. Then, a certain number of these dog tumors retrogress spontaneously. So his results were not at all conclusive.

I am very glad to hear that these experiments, so far as they have gone, agree with those done previously at Buffalo. The point that strikes me as most unsatisfactory in my own experiments is that they deal with tumor cells and not with tumors. They represent simply an effort to advance in the right direction. The final study should consist in making the injection of immune serum perhaps ten days after the intravenous inoculation of the tumor. This has not been done, and of course represents the crucial experiment as to the effect of immune serum upon implanted cells after they have once given rise to tumors.

In closing, however, I must again emphasize the fact that the final outcome of these experiments cannot be predicted from the preliminary observations, and that the complete results may eventually throw a different light on the problem.

### 19. Biological Effects of Radium on Tumor Cells

*Dr. Frederick Prime* (New York): There has been much discussion of late as to the penetrating power of radium rays and the efficacy of these rays in the treatment of malignant growths situated at some distance from the surface.

Two series of experiments were undertaken to determine the effect of radium on animal tumors. In the first, the tumor to be radiumized was removed and exposed in a moist chamber to the radium. At the end of the exposure, these pieces of tumor together with the control, which had been kept under similar conditions, were inoculated into mice or rats as the case might be. The tumors were charted at weekly intervals, and successive generations were propagated from the growths which appeared. It was found when the gamma rays were used, either in small or large doses, that after the first generation the tumors which had been exposed to small doses of radium for a short time produced larger daughter growths than did the controls, and larger also than those which had been exposed to a dose of radium just under the lethal point.

A second series of experiments was conducted upon tumors *in vivo*, a mouse carcinoma being used for this experiment. Here the mouse was anesthetized and the skin over the tumor reflected; then, after a small portion had been removed from the tumor and retained at the same temperature as a control, the radium, filtered through 0.4 mm. of brass, was applied to the tumor. This filter passes most of the beta particles except the softer groups. Exposures to 83 mgm. of radium element for two hours were made. After the application of the radium, some of the tumor was removed from the area immediately beneath where the radium had been, and at the same time tissue from the opposite side of the tumor, which was directly beneath the radium tube and never more than 1 to 1.5 cm. distant, was removed. Both of these pieces of tissue, together with the control tissue, were inoculated into a series of mice, and in some cases subsequent generations were carried on. After the first generation, the tumors arising from the tissue 1 cm. distant from the radium showed an increase in size as compared with the control, and with those grown from the tissue immediately under the radium. This stimulation effect often lasted through eight or nine generations of transplants.

From these experiments it is concluded: First, that the action of the gamma rays from radium in small doses upon tumor cells *in vitro* not only does not kill, but has a stimulating effect upon the cells, and that this effect persists through several series of generations of transplanted tumor.

Second, that the action of considerable quantities of radium upon tumor tissue *in vivo* is effective only after long exposure upon the tissue directly beneath the radium. There is often a stimulating effect upon tissue 1 cm. distant. This points to the necessity for employing very large quantities of radium, 1 gm. or more of the element, if any lethal effect is to be obtained on cells lying more than 1 cm. from the active agent.

DISCUSSION

*Dr. Henry Schmitz* (Chicago): I should like to ask Dr. Prime what length of time elapsed after the application of radium before the tumor was removed.

*Dr. Prime:* Immediately.

*Dr. Schmitz:* There is no immediately apparent effect after the use of radium, but there would be an effect two or three weeks later. It would be very interesting to continue these experiments, but to remove the tumors two or three weeks after the application of the radium and see what the results would be. The action of radium is latent.

*Dr. H. R. Gaylord* (Buffalo): I should like to ask Dr. Prime if he took into consideration the indirect effect of radium in these cases.

*Dr. James Ewing* (New York): I confess that in the brief time at disposal I could not convince myself that the conclusions drawn were justified by the charts. In many instances I saw but slight differences in the size of the tumors, and in some cases a favorable action by the radium. In other instances the experiments seem to bear out Dr. Prime's conclusions, but so far as I can see from a hasty observation of these charts, not invariably. I should like to study them more in detail before accepting the conclusions that Dr. Prime draws from his data.

Furthermore, it seems to me from the size of these tumors that the period of growth was hardly long enough to determine what the ultimate effect would have been, for all of the tumors shown in the chart were very small. Many of the controls hardly grew at all. I agree also with the first gentleman to discuss these results, that the experiments do not indicate what would happen to a tumor in the body after it has been treated by radium. If a growth be removed from the body immediately after such treatment, it is spared the effects of a number of intricate processes which occur in the tissues following exposure to radium. These constitute a very interesting, rather complex, and in some respects specific process, which enables one to recognize a tumor treated by radium after a week or two. Now, to remove such a tissue immediately after irradiation saves it from all the varieties of attack which nature can bring to bear by means of the blood serum, the leucocytes, and the connective tissue cells, and perhaps from still other processes which are not entirely clear.

The results are rather striking, also, in their variation from those of Hertwig, who showed that in order to stimulate protozoan nuclei it is necessary to use extremely small doses of radium. Beyond a certain amount he found an inhibiting influence upon nuclear mitosis. Again these findings reported by Dr. Prime are contrary to clinical experience in this matter; it was demonstrated as long as ten years ago that small

doses of radium accurately applied to superficial growths of the skin will cause complete disappearance of these tumors.

So, while the work is extremely important as suggesting a stimulating effect of radium, we ought to be very careful not to draw too broad conclusions. It seems possible that the stimulating effects may be referable to alterations in the tissue about incompletely treated tumors such as hyperemia and opening up of the tissue spaces. So far as I can discover from clinical work, that is the chief source of the so-called stimulating effects of radium upon tumor cells.

*Dr. F. C. Wood* (New York): I am sorry that Dr. Prime did not have a chart which would show a larger series of normal tumor controls. By comparing such a normal series in the same group of animals, the average weights of the tumors will be found to be very close, so I believe that the differences shown on the first chart will hold.

Dr. Ewing has referred to Hertwig's work. This has been much extended lately, and Hertwig himself has shown that if a cell in the resting stage be radiumized, stimulation will result, whereas if a cell in the dividing stage be radiumized with the same dose, either its growth will be slowed or it will be killed.

The value of Dr. Prime's experiments, it seems to me, lies in the fact that the tumor cells were removed from the body and that none of the secondary effects of tissue reaction were produced, the secondary effects being just what Dr. Prime wanted to avoid. It can now be generally accepted that the action of radium influences chiefly the nuclear mechanism of division; its effects do not appear until the cells have to divide. The primary effect of radium, however, occurs during the exposure, and it is important to avoid the secondary tissue effects on the tumor and to show that the stimulation which has been observed in sea urchins' eggs can be transferred to mammalian tissues.

## 20. The Refractory State Against Mouse Cancer Resulting from Large Doses of X-Ray

*Dr. B. T. Simpson* (Buffalo, N. Y.): In mice treated with a massive dose of X-ray from the Coolidge tube, the spleen was found most seriously damaged at the end of the second day; by the ninth the organ began to recover, as evidenced by increase in weight and the microscopic picture.

Arguing from the results which Murphy obtained with repeated small doses, i.e., the injury to the spleen with the consequent susceptibility of the body to the implantation of cells from foreign species, it was thought that tumor transplantation at the second day would show a large increase of positive results over the controls; by the fifteenth or twentieth day, on the contrary, when the spleen was recovered and even hypertrophied, it was thought that the percentage of takes would be decreased over the controls.

A series of experiments was undertaken to test this hypothesis, in

which the mice were exposed to the following dose of X-ray from the Coolidge tube: Fifty minutes, without filter, at a distance of forty-five centimeters, with twelve milliamperes and a six inch spark. These mice were inoculated with mouse adeno-carcinoma of the Jensen type at the second, fifth, tenth, fifteenth and twentieth day after exposure.

The outcome was quite contrary to our expectations, for at the end of the second day, where we expected to get a large percentage of takes, we had but 15 per cent, as against 60 per cent in the controls. At the fifth, tenth, and fifteenth days the inoculation outcome in the treated animals differed but little from that in the controls; at the twentieth day, there were 66 per cent of takes as compared with the controls, which gave 45 per cent.

The experiment was repeated with seventy mice, which were inoculated at the end of the second day after exposure, with 25 per cent of success as against 60 in the controls.

A series of experiments was then undertaken, in which an amount of spleen was injected comparable to that lost by the destruction following irradiation; the results of this series were negative.

Further experiments are being undertaken to try to find a satisfactory explanation of the phenomenon.

## 21. The Biological Effects of Radium Rays

*Dr. W. T. Bovie* (Boston): In connection with some investigations upon the biological effects of rays, it appeared to the writer to be desirable to make quantitative studies upon some physiological change caused by them. The conditions imposed by the investigation were exacting, and but few known physiological changes were suitable for measurement. It was hoped to find an easily observed physiological change fundamental to life processes, capable of being expressed quantitatively in terms of a normal control. It was necessary to make repeated observations on the same material. The time required for a single reading had to be short and one should be able to continue the experiment for two or three weeks. The change measured should be, as nearly as possible, the direct result of the radiation. The necessary technique should be such that it would be possible to compare various kinds of radiation, both alone and in combination with certain sensitizers and drugs.

This paper is a description of a method of studying a physiological change which fulfills the conditions enumerated above and which, it is hoped, will yield valuable results. Experiments are described for studying the effects of gamma and beta rays of radium emanation. The studies were made on the permeability of protoplasm to ions, as measured by its resistance to the passage of the electric current. This method for measuring permeability has been used by various investigators since 1897, and has been used most extensively in recent times by Osterhout. (In order to illustrate the method, curves were shown,

giving some of Osterhout's results upon the effects of various drugs upon protoplasm.)

Permeability has long been used as a measure of vitality, the usual tests being plasmolysis, penetration of dyes, and the diffusion of pigments. If permeability be measured by determining the resistance of the protoplasm to the electric current, then it becomes possible to express permeability, and consequently vitality, quantitatively. If we express vitality quantitatively, then we may speak of normal vitality, and it becomes possible to express the vitality of any mass of protoplasm at any time in terms of its normal vitality. (Curves were shown illustrating effects of the radium upon the vitality of protoplasm of *Saccharina* Laminaria, and it was shown that with the dosage used the effects were immediate, there being no perceptible latent period, although there was at first a slight lag in the effects of the rays. It was also shown that the decrease in vitality continued for a number of hours after the radium was removed, and it was pointed out that results seemed to indicate that for interrupted exposures the intensity of radiation times the time does not equal constant. That is, the effects are not strictly additive.)

No attempt is made in this paper to give a final discussion of the biological effects of the rays. The writer merely points out that the physiologist may, by studying the resistance of protoplasm to the electric current, measure the physiological effects of the rays, and thus bring to bear on his problem a mathematical treatment of the physiological changes similar to that made by the physicist when he uses an ionization chamber for measuring the intensity of radiation.

# THE EFFECTS OF CANCER TISSUE, EMBRYONIC TISSUE, AND NORMAL TISSUE ON THE VITALITY OF PROTOZOA

## DIDINIUM NASUTUM. III

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Experiments begun in the winter of 1914–15 indicated the presence in cancer tissue of lethal and stimulating factors which affect the vitality of *Didinium nasutum* a carnivorous ciliate.[1] It was found that cancer tissue has a well-marked effect on the vitality of *Didinium* as shown by differences of the division rates of the cancer-treated series over those of the parallel series of control animals.

The lethal effects, shown in the series treated with single and double doses of cancer, were indicated by reduced division rates and increased death rates as compared with the controls. With the single dose the average division rate for the period of fifteen days' treatment was 1.84 divisions per day per individual while that for the control series was 2.48 per day. With the double dose, the division rate was 1.38 per day while that of the control was 2.55. The death rate for the single dose series was the same as that for the control, but in the double dose series it rose to 40 per cent of all the animals treated as against 16 per cent for the control. Further evidence of the lethal factor was shown by the *after effects* on the vitality of organisms kept in a normal medium for forty-eight hours after continuous treatment in 5-day periods. In the series that had been treated with a double dose of cancer, the death rate during these periods was 100 per cent.

[1] The Effects of Cancer Tissue and Normal Epithelium on the Vitality of Protozoa. *Didinium nasutum II.* Jour. Cancer Research, 1916, I, 205.

The stimulating effect of cancer tissue was masked by the activity of the lethal factor in the single and double dose series, but was apparent in the half dose series. Here the average division rate was 2.67 divisions per day per individual as against 2.55 for the control, while the death rates were 14.6 per cent and 16 per cent respectively. Further evidence of the stimulating factor was seen in the after effects without cancer in the half dose and the single dose series. The division rate of organisms that had been treated with the half dose rose to 2.93 while the control was 2.50, with death rates of 0 per cent and 46.6 per cent respectively. The division rate of organisms that had been treated with a single dose rose to 3.26 with a death rate of 0 per cent while the controls for this series had a division rate of 2.59 and a death rate of 53.3 per cent. The high death rate of the controls in these series was due to the fact that the animals were supplied with food sufficient for only one day, so that conditions of starvation characterized the last twenty-four hours of the forty-eight hour periods. Exactly the same conditions, however, were maintained in all series that had been treated with cancer, so the high death rate of the controls and the entire absence of mortality in the cancer-treated individuals show that the latter had been stimulated and were better able to withstand adverse conditions than the control animals from which they were derived.

Still further evidence of stimulation was found in the behaviour of *Didinium* during the first five hours subsequent to feeding and treatment with cancer. During such periods for the entire course of the experiments, only 25 per cent of the controls divided, while 53 per cent of the half dose cancer-treated individuals, 43 per cent of the single dose, and 45 per cent of the double dose cancer-treated individuals divided.

A second set of experiments was similarly carried out to see if normal epithelium, prepared in the same way as the cancer tissue, would show similar lethal and stimulating effects on *Didinium*. The results gave unmistakable evidence of a stimulating factor but no evidence at all of a lethal factor, the death rates for the half, single, and double dose series being 2.6 per

cent, 4 per cent, and 6.6 per cent respectively, while the death rate of the controls was 10.6 per cent. The division rate of the single dose series was 2.79 per day and for the double dose series 2.64, while the control was 2.67. The half dose series averaged 2.95 and its control 2.79. The high division rate of all series at this time was due to a recent period of rejuvenescence with general high vitality, so that the differences in the division rates have a greater significance than the figures indicate.

Stimulation was also shown in the after effects. During the forty-eight hours under observation, of which the last twenty-four were under conditions of starvation, the controls divided on the average 2.82 and 3.27 times while the death rate went to 26.6 per cent. At the same time, the series which had been continuously treated with the half, single, and double dose, divided 3.00, 3.27, and 3.55 times respectively, with death rates of 6.6 and 0 and 0 per cent.

Similarly, the records for the first five hours after treatment showed an increase in the division rate of the tissue-treated animals over that of the control, giving additional evidence of the stimulating effect of treatment with normal epithelium.

The general results of the experiments led to the conclusion, already reached by Woodruff and Underhill, that cancer tissue contains some substance or substances that have a depressing effect on the vitality of normal, free-living cells, and something, also, which acts as a stimulant. The results show, furthermore, that normal adult skin contains a similar stimulating factor without any evidence of the lethal factor.

The experiments were repeated during the winter and spring months of the present year to see if another type of tumor would give similar results. For these a rat tumor and rat skin were used in place of the mouse tumor and mouse skin employed in the first experiments. The methods adopted throughout were the same as those of last year, but a different race of *Didinium nasutum* was used for controls and indicators, for which we are indebted to Dr. S. O. Mast, of Baltimore, Md.

Four sets of experiments were carried out; one with the Flexner-Jobling rat carcinoma, one with adult rat skin, one with rat

embryo skin, and one with rat embryo skin autolysates. Each set was continued for three consecutive periods of five days each, with two-day intervals between the periods for testing the after effects. The cancer and normal skin sets were carried on at the same time from February 28 to March 20, and the embryo skin and embryo autolysates sets from March 20 to April 10.

## 1. MATERIALS AND METHODS

*Didinium nasutum* is a fairly large infusorian easily seen and manipulated with a fine pipette under a low power binocular. It normally feeds on *Paramecium*, which is swallowed whole. In the culture experiments, a single individual is transferred to a square ground-glass dish 40 mm. by 8 mm., containing 0.5 cc. of Great Bear spring water and ten *Paramecium caudatum* (last year nine *Paramecium* were used). Four other similar culture dishes are prepared in the same way and stacks of five are stored in glass moist chambers. These contain the control animals from which sister individuals are taken for the tissue experiments. For these the culture dishes are prepared as above, one *Didinium* and ten *Paramecium* in each, the sole difference from the control being the addition of the tissue to be tested. As before, three dosages of each tissue were used, which are designated the half, single, and double dose, care having been taken to make the dosage as uniform as possible from day to day. For each dose employed, five individuals of *Didinium* were isolated, fed, and treated daily.

Forty lines of *Didinium* were thus carried on continuously for the entire period of the experiments. All were recorded, transferred to fresh water, and fed with ten *Paramecium caudatum* each, every morning between 9 and 11 o'clock, the tissue being added last to the culture dishes. At 4 p. m. every watch glass was examined and records were made of the number of divisions of *Didinium*, the number of living *Paramecium*, and the number of deaths and encystments, if there were any. These five-hour records are important in connection with evidence of initial stimulation. Similar records were made every morning twenty-

four hours after feeding. If two individuals of *Didinium* were present, a record of one division was made; if four or eight were present, two or three divisions were recorded. A record was also made of the number of living *Paramecium* present in the culture dish. Every morning, also, one individual *Didinium* was transferred from each of the culture dishes to fresh water, and given fresh food and the appropriate tissue treatment. In case of death in any culture dish, an individual from another line of the same series was isolated in its place, and in case of death in every line of a series their places were filled from the next lower dosage series.

At the end of five days of treatment (Monday to Saturday) single individuals from all lines were isolated as before in fresh water without tissue. Each was given ten *Paramecium* and left for forty-eight hours. The food given was enough for only one day, so the second day was a period of adverse condition of starvation, and the records at the end of the forty-eight hour period serve as an index of vitality of the organisms that had been variously treated the week before. These records are used in determining the after effects of treatment.

The standard measure of protozoon vitality is the division rate. This was determined for each five-day period by adding the total number of divisions and dividing by the number isolated in each series during the period. The percentage of deaths was worked out in the same way, and the figures thus obtained form the basis of the accompanying tables.

In order to ensure as uniform cancer material as possible, rats were inoculated with the same tumor strain at such times as to give a fresh twenty-day tumor for each day of the experiments; on one day only, the last, was it necessary to use a twenty-one day tumor.

## 2. EFFECTS OF CANCER TISSUE

Aseptic precautions were taken in removing the tumor from the rat and in preparing the emulsion. The healthy tissue was separated from the necrotic part and emulsified in a sterile glass dish by repeated cutting with curved scissors. The mush thus

prepared was used immediately, the dosage being measured by a fine platinum loop. No odor of putrefaction was apparent in the watch-glasses during the twenty-four hours of treatment, the bacteria being kept down by the living *Paramecium*. The general results for the fifteen days of treatment, and the after effects, are shown in table 1.

TABLE 1

*Effects of rat cancer mush*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| | | FIRST PERIOD | SECOND PERIOD | THIRD PERIOD | AVERAGE 15 DAYS | CORRECTED AVERAGE | FIVE HOUR RECORDS | AFTER EFFECTS | |
| | | | | | | | | Divisions | Deaths |
| Control. | Division rate... | 1.32% | 1.84% | 1.54 | 1.57 | 1.66 | 32.0% | 2.33 | 6.6% |
| | Death rate..... | 6.0% | 6.0% | 4.0% | 5.3% | | 6.0% | | |
| Half dose .. | Division rate... | 1.24 | 1.28 | 2.08 | 1.53 | 1.56 | 34.6% | 2.53 | 13.3% |
| | Death rate..... | 8.0% | 12.0% | 0.0% | 6.6% | | 4.0% | | |
| Single dose .. | Division rate... | 0.60 | 1.00 | 1.08 | 0.89 | 1.15 | 25.3% | 1.66 | 26.6% |
| | Death rate..... | 44.0% | 60.0% | 40.0% | 48.0% | | 16.0% | | |
| Double dose .. | Division rate... | 0.88 | 0.80 | 0.80 | 0.83 | 1.08 | 18.6% | 1.73 | 40.0% |
| | Death rate..... | 48.0% | 48.0% | 56.0% | 50.6% | | 16.0% | | |

Except for the absence of any indication of stimulation, these results with the rat carcinoma agree with those obtained last year with mouse tumor. The lethal factor is evident, as shown by the increasing death rate with increased dosage (from 5.3 per cent in the control to 50.6 per cent in the double dose series—column 6), as well as by the decreasing division rate, which is shown best in column 7, where the figures are based only on the *Didinium* which were not killed by the treatment. The effect of the lethal factor is also shown in the after effects (columns 9 and 10), where the death rates in the cancer-treated series run from two to nearly seven times that of the control series.

The division rate of the half dose series ran behind that of the control for the first ten days and well ahead during the last five days, so that the net result is an average rate practically the same as that of the control. There may be some evidence here of an acquired immunity. With the mouse tumor experiments of last year, the half dose series showed unmistakable evidence of stimulation, both in higher division rate and lower death rate than the control. In the present experiments, the only evidence of stimulation is seen in the slightly higher division rate, in the after effects, and in the records for the first five hours after treatment (column 8). The lethal factor, however, is so strong in this cancer that its effects are apparent during the first five hours after treatment, where, with the exception of the half dose series, the decreased division rate and the increased death rate for both the single dose and double dose cancer-treated series show the depressant effect of the cancer.

### 3. EFFECTS OF NORMAL SKIN TISSUE

Healthy young rats from three to five months old, free from parasitic skin diseases, were selected. The hair was shaved from the abdomen and side of the rat and a strip of the shaved skin was removed and washed in running water. It was then wiped dry and spread out upon a clean glass plate and the under surface was thoroughly scraped with a scalpel to remove the greater part of the connective tissue. The outer surface was then gently scraped to remove the keratin. After several washings in spring water, the thin strip of tissue was placed in a small glass dish and a few drops of spring water and one or two drops of the rat's blood were added. The tissue was then reduced to a fine emulsion by cutting with scissors, thorough cleanliness being observed throughout this and the earlier procedures. These operations were repeated daily during the entire period of the experiment. The mush thus prepared was used mmediately in three dosages, these dosages being increased proportionately as the experiment progressed, as no lethal factor was evident. The general results are shown in table 2.

TABLE 2

*Effects of adult rat skin*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| | | FIRST PERIOD | SECOND PERIOD | THIRD PERIOD | AVERAGE 15 DAYS | CORRECTED AVERAGE | FIVE HOUR RECORDS | AFTER EFFECTS | |
| | | | | | | | | Divisions | Deaths |
| Control. | Division rate... | 1.32 | 1.84 | 1.54 | 1.57 | 1.66 | 32.0% | 2.33 | 6.6% |
| | Death rate..... | 6.0% | 6.0% | 4.0% | 5.3% | | 6.0% | | |
| Half dose .. | Division rate... | 1.52 | 2.00 | 1.68 | 1.73 | 1.73 | 32.0% | 3.00 | 0.0% |
| | Death rate..... | 0.0% | 0.0% | 0.0% | 0.0% | | 0.0% | | |
| Single dose .. | Division rate... | 1.52 | 1.96 | 1.60 | 1.69 | 1.70 | 40.0% | 2.73 | 6.6% |
| | Death rate..... | 0.0% | 0.0% | 4.0% | 1.3% | | 0.0% | | |
| Double dose .. | Division rate.. | 1.40 | 1.96 | 1.28 | 1.55 | 1.70 | 36.0% | 1.86 | 0.0% |
| | Death rate..... | 12.0% | 0.0% | 24.0% | 12.0% | | 8.0% | | |

These are very different results from those obtained with rat cancer, carried on at the same time and with the same controls. The death rates and division rates show the absence of a lethal factor except, possibly, in the double dose series. Here, however, the high death rate (24 per cent) in the last five day period was probably due to the excess of tissue used, where, in the last period the dosage ran up to two and three times that of the double dose cancer series. The absence of the lethal factor is also shown in the after effects, where the death rate was never in excess of that for the control, while for the strongest and the weakest doses it was nil.

Evidence of a stimulating factor, on the other hand, while not pronounced, was nevertheless evident, as shown by the corrected division rates, the five-hour records, and the after effects. These results confirm in all respects, therefore, the results with adult mouse tissue obtained last year.

It is quite evident from these experiments that typical, rapidly growing cancer tissue has a different effect on the living indicator

than has normal adult epithelium. This is particularly clear in connection with the so-called lethal factor, no evidence for which can be found in the adult normal tissue used. The nature of the lethal factor is a matter of considerable interest and at the present time can only be a subject for speculation. In regard to the origin of the lethal substance or substances, there appear to be only two alternatives; first, they may be products of the abnormal growth, i.e. of the tumor as a whole, or, second, they may be products of rapidly growing cells and quite independent of the malignant growth. In the earlier publication (loc. cit.) the suggestion was made that the necrotic material of cancer may be the source of the lethal factor through the formation of toxic substances. To test this, we emulsified the necrotic portions of the rat cancers used during the last five-day period of the cancer experiment. As with the cancer and normal skin material, this was used in three dosages given to three series of five *Didinium* each for a period of five days. The records, averaged for the five-day period, are summarized as follows:

| | DIVISION RATE | DEATH RATE | AFTER EFFECTS | |
|---|---|---|---|---|
| | | | Divisions | Deaths |
| | | *per cent* | | *per cent* |
| Control | 1.54 | 4.0 | 2.30 | 20 |
| Necrosis. Half dose | 1.56 | 0.0 | 3.00 | 0 |
| Necrosis. Single dose | 1.12 | 16.0 | 2.60 | 0 |
| Necrosis. Double dose | 0.96 | 28.0 | 1.80 | 40 |

A comparison of these results with those in tables 1 and 2 shows a closer agreement with the effects of cancer tissue than with normal epithelium. A lethal effect is unmistakable, as shown both by the division rate and the death rate in each of the higher dosage series. The results under "after effects," while interesting, are not conclusive, owing to the small number of animals under observation in each set (five).

While the depressant effect of the necrotic material was well marked, especially with the stronger doses used, it was not strong enough to account for the still more emphatic effect of

the cancer tissue from which the necrotic portions were carefully removed in making the emulsion. Similar lethal effects with embryonic tissue, in which there can be no question of products of necrosis, show that the necrotic portions of cancer are not responsible for the depressant effect on *Didinium*.

### 4. THE EFFECTS OF RAT EMBRYO SKIN TISSUE

Unborn rats in the late stages of embryonic growth were the sources of material for these experiments, and fetuses varying from a few hours to several days before birth were chosen, since they offer no difficulties in the removal of the skin. Fresh embryos were used every day for the entire period of fifteen days, except on one or two occasions when scarcity of embryos compelled us to use material of the day before, kept in cold storage. In each case the fetus was removed by the usual aseptic methods and placed in a sterile dish, where the skin was readily stripped off with forceps. The tissue was then reduced to a fine mush with curved scissors. As in the previous experiments, three dosages were used on sister cells of the control *Didinium*.

A similar set of experiments with mouse embryos was carried out in May, 1915, when the vitality of the race of *Didinium* used was extremely low owing to a period of depression. Also, the temperature was high, so that putrefaction was an added difficulty in the interpretation of results. The results, however, were interesting and may be summarized as follows: With the half dose the division rate was higher on twelve and lower on three days than the control, while the death rate was 17.3 per cent as against 30.6 per cent for the control. With the single dose, the division rate was higher on eight days and lower on seven days than the control, with death rates of 28.0 per cent and 30.6 per cent respectively. With the double dose the division rate was higher than that of the control on seven days and lower on eight days while the death rates were 42.0 per cent and 30.6 per cent respectively. These results, while not positive, give some evidence of stimulation with the weaker doses used, and some evidence of a lethal factor with the strongest dose.

The present experiments with embryo skin were carried out under more favorable conditions. The *Didinium* indicator was normal and the temperature from March 20 to April 10 was uniformly cool, so that putrefaction was avoided. The results, as shown in table 3, leave no ground for uncertain conclusions.

TABLE 3

*Effects of rat embryo skin*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| | | FIRST PERIOD | SECOND PERIOD | THIRD PERIOD | AVERAGE 15 DAYS | CORRECTED AVERAGE | FIVE HOUR RECORDS | AFTER EFFECTS | |
| | | | | | | | | Divisions | Deaths |
| Control. | Division rate... | 1.48 | 1.62 | 1.94 | 1.68 | 1.85 | 38.0% | 1.96 | 26.6% |
| | Death rate..... | 12.0% | 6.0% | 6.0% | 8.0% | | 4.6% | | |
| Half dose.. | Division rate... | 1.44 | 1.72 | 1.92 | 1.69 | 1.70 | 28.0% | 1.93 | 20.0% |
| | Death rate..... | 4.0% | 4.0% | 0.0% | 2.6% | | 0.0% | | |
| Single dose.. | Division rate... | 1.20 | 1.92 | 1.20 | 1.44 | 1.75 | 38.6% | 2.46 | 13.3% |
| | Death rate..... | 28.0% | 12.0% | 44.0% | 28.0% | | 2.6% | | |
| Double dose.. | Division rate... | 0.48 | 1.52 | 0.64 | 0.88 | 1.31 | 25.3% | 1.00 | 66.6% |
| | Death rate..... | 48.0% | 16.0% | 36.0% | 33.3% | | 4.0% | | |

There is little evidence of a stimulating factor in this embryonic tissue, the only possibility being the increased division rate and the decreased death rate in the after effects of the single dose series, which, by itself, means little. On the other hand, there is abundant evidence of the presence of a lethal factor, shown in relation to the control by (1) the high death rate in the two stronger dosage series; (2) the lower division rate in all series; (3) the lower division rates in the five hour records, and (4) the lower division rate and the high death rate in the after effects of the double dose series. The results as a whole, resemble those for the cancer tissue experiments rather than those for the adult skin (compare tables 1 and 2, also table 5).

### 5. THE EFFECTS OF RAT EMBRYO AUTOLYSATES

A fourth set of experiments, to see if embryo tissue autolysates have a stimulating effect on the living indicator, was carried on at the same time as the embryo tissue experiments. The autolysates were prepared from the emulsified material as used for the embryo experiments. Sterile pipettes were partially filled with the emulsion, both with and without the addition of sterile spring water, and the ends of the pipettes were then sealed in the flame. These pipettes were then placed in an incubator at 37°C. for forty-eight hours (first and second periods) and for from five to nine days (third period). A fresh tube was opened each day and the contents distributed as in the other experiments, three dosages being given to five lines each of *Didinium*. The results are tabulated in table 4.

TABLE 4

*Effects of rat embryo autolysates*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| | | FIRST PERIOD | SECOND PERIOD | THIRD PERIOD | AVERAGE 15 DAYS | CORRECTED AVERAGE | FIVE HOUR RECORDS | AFTER EFFECTS | |
| | | | | | | | | Divisions | Deaths |
| Control. | Division rate... | 1.48 | 1.62 | 1.94 | 1.68 | 1.85 | 38.0% | 1.96 | 26.6% |
| | Death rate..... | 12.0% | 6.0% | 6.0% | 8.0% | | 4.6% | | |
| Half dose.. | Division rate... | 1.48 | 2.08 | 1.88 | 1.81 | 1.89 | 38.6% | 2.00 | 13.3% |
| | Death rate..... | 8.0% | 0.0% | 8.0% | 5.3% | | 1.3% | | |
| Single dose.. | Division rate... | 1.36 | 2.04 | 1.80 | 1.73 | 1.88 | 38.6% | 1.60 | 33.3% |
| | Death rate..... | 20.0% | 4.0% | 8.0% | 10.6% | | 0.0% | | |
| Double dose.. | Division rate... | 0.72 | 1.52 | 1.00 | 1.08 | 1.33 | 29.3% | 1.06 | 46.6% |
| | Death rate..... | 56.0% | 8.0% | 28.0% | 30.6% | | 5.3% | | |

The half dose series and the single dose series show some evidence of stimulation but the double dose series shows a marked depressant effect in all of the records. This experiment is

worth repeating with weaker doses than those used, for the results give reason to believe that the stimulating factor would be more effective under such conditions. The length of time allowed for autolysis appeared to make little difference in the results. In the first two periods (columns 3 and 4) the tissue was allowed to autolyze for forty-eight hours only, while in the third period (column 5) autolysis lasted from five to nine days. The average division rates are intermediate between those of the first two periods for all doses employed.

### 6. GENERAL

The relative effects of all tissues used in these experiments are shown in summarized form in table 5.

The corrected division rates represent the division energy of the organisms experimented with, and do not include those that died during the period of the experiments. The averages given represent the effects, if any, of the treatment on metabolism of the free cells. The uncorrected division rates, on the other hand, indicate the general vitality of all the organisms isolated and treated, and here the variations, as would be expected, are more pronounced. In either case the death rates must also be considered in order to interpret the results correctly. The five-hour division rates are interesting, as showing the presence or absence of initial stimulation after treatment, although these results are not so striking as those obtained last year. The after effects likewise are interesting, as showing the lasting effects in regard to improved or impaired vitality.

The general results of the experiments confirm the conclusion reached last year, that a lethal factor is present in cancer tissue which is absent in normal tissue. The stimulating factor, however, is either not present in this tumor, or else its effects are masked by the more potent lethal factor. Some evidence of it is seen in the increased division rate in the five-hour records and in the after effects of the half dose cancer series, but these are not well marked. The death rates, on the other hand, show a very pronounced lethal factor, 48 per cent and 50 per cent

for the single and double dose series as against 5 per cent for the control, whereas in 1915 the rates for the same series were 16 per cent and 40 per cent as against 16 per cent for the control. A similar depressant effect is shown in all of the other records

TABLE 5

*Summarized results for all tissues used*

| RECORDS AVERAGED FOR 15 DAYS | | ADULT SKIN TISSUE | | | | CANCER TISSUE | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | Half dose | Single dose | Double dose | Half dose | Single dose | Double dose |
| 24 hour division rates | Corrected | 1.66 | 1.73 | 1.70 | 1.70 | 1.56 | 1.15 | 1.08 |
| | Uncorrected | 1.57 | 1.73 | 1.69 | 1.55 | 1.53 | 0.89 | 0.83 |
| Death rates | | 5.3% | 0% | 1.3% | 12% | 6.6% | 48% | 50% |
| Five hour records | Divisions | 32% | 32% | 40% | 36% | 34.6% | 25.3% | 18.6% |
| | Deaths | 6% | 0% | 0% | 8% | 4% | 16% | 16% |
| After effects | Divisions | 2.00 | 3.00 | 2.73 | 1.86 | 2.53 | 1.66 | 1.73 |
| | Deaths | 6.6% | 0% | 6.6% | 0% | 13.3% | 26.6% | 40% |
| | | EMBRYO SKIN TISSUE | | | | AUTOLYSATES | | |
| 24 hour division rates | Corrected | 1.85 | 1.70 | 1.75 | 1.31 | 1.89 | 1.88 | 1.33 |
| | Uncorrected | 1.68 | 1.69 | 1.44 | 0.88 | 1.81 | 1.73 | 1.08 |
| Death rates | | 8% | 2.6% | 28% | 33.3% | 5.3% | 10.6% | 30.6% |
| Five hour records | Divisions | 38% | 28% | 36.6% | 25.3% | 38.6% | 38.6% | 29.3% |
| | Deaths | 4.6% | 0.0% | 2.6% | 4.0% | 1.37 | 0.0% | 55.3% |
| After effects | Divisions | 1.96 | 1.93 | 2.46 | 1.00 | 2.00 | 1.60 | 1.06 |
| | Deaths | 26.6% | 20% | 13.3% | 66.6% | 13.3% | 33.3% | 46.6% |

of the single and double dose cancer series, whereas analogous records of last year frequently show evidence of stimulation.

The most significant of the results obtained were those in connection with the fresh rat embryo skin. Here, no less than

with the cancer series, the lethal effect is unmistakable. This is shown by the diminished division rates in the five-hour, twenty-four hour, and "after effects" records, and in the death rates of the larger dosage series throughout. The series of embryo tissue experiments, as a whole, resembles that for the cancer tissue, the depressant effect being somewhat less pronounced. Nor is there any more evidence of a stimulating factor than in the cancer series, the half dose series alone running close to the control.

Analogous results were obtained with the embryo skin autolysates, with some slight evidence of stimulation in the smaller dose series.

It is obvious that the depressant effect cannot be due to the products of necrosis, since the embryo series gives the same result as the cancer series. Furthermore, the absence of all evidence of a lethal factor in the adult skin series indicates that the lethal factor of embryo skin is lost with the adult condition. Characters common to cancer and embryo skin are not numerous, although cancer cells are frequently described as manifesting some of the properties of embryonic cells. The one important common property is that of rapid cell division with accompanying more active cellular metabolism. Adami[2] makes the statement: "Undoubtedly the trend of recent work is to show that malignant tumors excrete or afford substances, some of them of the nature of enzymes, which are of toxic nature, and it is a reasonable view that these tell especially upon the immediately surrounding tissues. . . ." If such toxins from cancer are the cause of the depressant effect on *Didinium*, are similar toxins from embryonic skin tissue the cause of analogous depressant effects? If they are the same, their origin cannot be from degenerating cells but rather from normal cells which are in process of active cell division, since the adult skin cells show no such toxic effect. It follows, therefore, that the lethal factor for *Didinium* is not due to necrosis, but to some factor or factors of living, highly metabolic cells. In cancer it is possible, as

[2] Adami, Principles of Pathology, vol. I, p. 776.

Adami suggests, "that these tell especially upon the immediately surrounding tissues" and lead to degeneration of cells in such tissues. But products of degeneration of normal cells have a stimulating effect on *Didinium* and presumably on normal tissue cells, which in turn would respond by dividing, and a vicious circle would thus be set up, the influence of which would extend to the point where lethal and stimulating factors are balanced.

# OBSERVATIONS ON THE MODE OF ORIGIN OF THE FIBROADENOMA OF THE MAMMARY GLAND IN THE RAT AND ON THE DELAYED RETROGRESSION OF THE MAMMARY GLAND AFTER THE PERIOD OF LACTATION

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Our previous observations (1) prove that the fibroadenoma of the mammary glands is a typical new formation in rats, which is of considerable interest in the analysis of tissue and tumor growth. It is, therefore, of importance to record data which tend to throw some light on its mode of origin. The following observations represent a first attempt in this direction. At the same time they give some information about the processes which lead to the retrogression of tissues that have proliferated; they show in particular how a delayed retrogression of the mammary gland may be compensated for, and present, furthermore, an interesting analogy with certain cirrhotic conditions found especially in the liver.

A female rat from a stock inbred for eleven generations was received from Granby, Massachusetts, having been sent there from the Wistar Institute in Philadelphia. She was about twenty to twenty-one months old and had given birth to three young ones about three to three and one-half months previously. She had suckled the young, which had developed in a normal manner. On examination, it was found that the mother had four tumor-like masses at the site of the mammary glands. One was situated posterior to the right front leg; on cutting this was found to be a cyst filled with rather thick yellow fluid, which represented apparently autolysed material. No putrefaction had

taken place. The cyst measured approximately 8 by 10 mm. in two diameters. In the region toward the right hind leg was found a larger cyst, measuring approximately 10 by 20 mm., which contained clear fluid. On the same level with the latter, on the left side of the animal, was a similar cyst, and still further caudad, in the region of the most posterior left mammary gland, there was a solid mass instead of a cyst. The diameters of the cyst and of the solid mass on the left side were intermediate between those of the two cysts on the right side. Otherwise the animal appeared normal. The ovaries were of the average size, the uterus fairly large but within the limits of normal variations.

### MICROSCOPIC EXAMINATION

1. The microscopical examination of the solid gland showed the following. The tissue has a lobulated arrangement. As a rule, dense fibrous tissue concentrically surrounds the acini or ducts. There is a fairly equal distribution of acini or ducts. In some areas, however, they are much more frequent than in others. In some of the ducts or acini colloid material is present. The gland structures consist of medium size cells with vesicular nuclei, some of the cells being vacuolated like those of the secreting mammary gland. One mitosis is seen in an epithelial cell. Sometimes these vacuolar cells form strands, at other places solid alveoli in the dense connective tissue. At some places there are both acini and ducts as well as solid alveoli of vacuolar cells present. The strands of hyaline fibrous tissue contain well formed vesicular nuclei or elongated compressed nuclei. Yellow pigment is frequently present in the fibrous tissue. In places, the lumen of the ducts is widened, and irregular prominences of connective tissue lined by epithelium intrude into the lumen. It seems in certain areas as though the connective tissue separating acini with vacuolated cells had disappeared and that nests of vacuolated cells had thus been produced. Or the ducts may be bent in various curves, and thus join to form alveoli.

2. The structure of the three small cysts is essentially the

same. We do not, therefore, need to describe them separately. They are lined by low cuboidal epithelium, as a rule, though in some areas the epithelium is higher, and occasionally it forms several rows of cells. There are some mitoses visible in the epithelium but on the whole they are rare. The epithelial covering is not everywhere complete, but there are defects which are not yet covered by epithelium. At such places, adherent to the underlying connective tissue, there is usually a polyp-like mass consisting of unprotected connective tissue, which shows necrosis and solution in progressive stages from the cyst wall toward the lumen of the cyst. The epithelium covers a connective tissue wall composed of well developed, dense fibers. Blood-vessels with a simple endothelial lining traverse the connective tissue. At some places the connective tissue cells swell, become epithelioid, and often contain yellow pigment. Occasionally, there are hemorrhages in such areas. The yellow pigment in the connective tissue cells is probably transformed haemoglobin. At other places, connective tissue cells may become swollen without containing pigment. Between such swollen cells connective tissue fibers are still present. In one area, the epithelium has regenerated over such enlarged connective tissue cells, and epithelial cells have sent processes downwards which surround some of the connective tissue cells. It is especially at such places, where the connective tissue cells are enlarged, that the epithelial covering is incomplete, and that the attachment of tissue that is undergoing necrosis to the underlying connective tissue can be observed. There, too, capillary blood-vessels are seen growing out into the polypous excrescences; the blood-vessels are surrounded by the swollen connective tissue cells, between which connective tissue fibers can be distinguished. The tissue is at first well preserved. Gradually the connective tissue fibers swell or disappear, the cells take up still more water, and the blood in the capillary vessels clots and forms a homogeneous mass. The vesicular nuclei of the connective tissue cells resist longest and can still be recognized when cells, fibers, and blood-vessels as such are no longer distinguishable. In the end the

whole necrotic material forms one gelatinous mass, which stains red with eosin The loss of structure is gradual and complete in the center of the cyst, where we find a loose material staining red with eosin. The solution of the necrotic material which takes place in all three cysts is particularly marked in two of them. In one of the cysts the retained material is still somewhat more consistent. While at many places the connective tissue cells swell before undergoing degeneration, and form a mass of large polygonal cells which seem to cause compression and absorption of the connective tissue fibrils between them, at other places the fibrous tissue as such swells over a wide area and in the end forms a structureless mass.

At various places polynuclear leucocytes migrate through the cyst walls into the necrotizing and necrotic central mass. The number of admixed leucocytes is usually considerable. Toward the center the leucocytes become dissolved, together with the other material. At certain places in the periphery of the necrotic material we find collections of threads which stain blue with hematoxylin; they somewhat resemble mycelia, but it can be shown that they take their origin in connective tissue nuclei which are compressed through the swelling of the connective tissue fibers and are thus drawn out into threads. They also are dissolved as we approach the center of the necrotic material. In the connective tissue wall of the large cyst we find smaller cysts in which the same processes are going on; the tissue is breaking down and the epithelium regenerates around the cavities thus formed. Those smaller cysts join with the large central cyst and thus the wall of the large cyst is covered by papillomatous formations, which show the same structure as the wall of the cyst. Everywhere we find in the periphery of the cyst, in the smaller adjoining cysts (which are probably all connected with the lumen of the main cyst), tissue that still maintains some resemblance to the normal tissue, showing connective tissue and blood-vessels, and here we find much of the dying tissue infiltrated with polynuclear leucocytes, while toward the center the material becomes a structureless mass which takes up more and more water and stains

with eosin. At one place our sections show a connection between one large cyst and the skin; it probably corresponds to the opening of a mammary duct.

Some places in the walls of the cysts present collections of small acini of mammary glands; these acini usually have low epithelium and a narrow lumen.

These then are the facts. How shall we interpret them?

In this animal, which had had young ones three to three and one-half months ago, the normal retrogression of the mammary glands failed to take place. The cause for this failure must probably be sought in some factor affecting the animal as a whole and not in a mere accidental local change, because we find this failure in four different glands simultaneously. In place of retrogression, we find large portions of the gland tissue undergoing necrosis. In our case we could actually see this breaking down as it affects the connective tissue and blood-vessels, but it may have previously involved gland tissue proper as well. Through the breaking down of the tissues, ducts are opened and the neighboring epithelium begins to regenerate and to separate the healthy from the dying tissue. It is probable that in the neighborhood of the larger vessels and in the periphery of the gland the tissue remains best preserved, while the breaking down begins in the central parts of the gland. At first the tissue that is destined to die merely takes up much water, but then gradually disintegrates, undergoes autolysis, until it becomes in the end more and more a fluid mass. The necrotic tissue at the same time attracts polynuclear leucocytes. This process continues for some time; while the material in the center liquefies more and more, new tissue breaks down at the periphery. Thus a large cyst is formed through regeneration of the epithelium of the large ducts. This cyst in the end communicates with the lumen of the large ducts. Evidently the fluid in the cyst became stagnated.

In this case we are evidently dealing with a process comparable to the necrosis of the deciduomata after the growth stimulus has ceased to act on them. They become necrotic en masse, autolyse, and are absorbed. A total necrosis likewise takes

place in the placenta after delivery, but in this case the necrotic tissue soon separates from the underlying healthy tissue and is cast off. Here also, as well as in the case of necrotic deciduomata, the epithelium of the uterus regenerates underneath the dying tissue and helps to separate it from the living healthy mucosa.

While these changes took place in three of the mammary glands, in the fourth the breaking down of the tissues did not occur. Instead, the greater part of the gland lost the character of secreting gland tissue and returned to that of an ordinary functionally inactive gland in which at the present time only very little, if any, growth is taking place. Here and there, however, there are some remnants of gland tissue in which the cells show the vacuolar character of secreting cells. The connective tissue surrounding this gland tissue is dense. We have here to deal with a structure which in all essentials is like the adenofibromata that we have studied experimentally and on which we reported in two other papers. We found that these adenofibromata may be to a certain extent transplantable, and that even in the third generation proliferative processes may become apparent, which are not found in normally growing glands, and while not yet indicative of malignant growth, at least represent a transition to abnormal growth processes.

We may conclude that in all probability the failure of a mammary gland to retrogress in the usual manner after the end of lactation is the cause of the formation of an adenofibroma of this gland in the rat. We may furthermore assume that there are two conditions responsible for the origin of the adenofibroma: (1) general conditions which prevent the normal retrogression of a number of mammary glands, and (2) a special local condition which prevents the breaking down of this gland and leads instead to the preservation and increase of its gland and connective tissue structures. Concerning the character of these two kinds of factors we can make no definite statement, but we need not necessarily assume that this local factor was of a very specific nature; there is no reason for believing that a micro-organism was the cause of this difference in the behavior of the fourth

gland; some slight accidental modification of some unknown kind might account for this difference.

Our observations are of interest in still another way. The adenofibromatous condition of the mammary gland corresponds in certain respects to a cirrhosis. We may find a parallel development of connective tissue and of epithelial elements, and a hypertrophy of the whole organ, as in the case of the liver.

Now it is usually assumed, in accordance with Weigert's views, that hypertrophic proliferation of connective tissue is invariably the result of primary destruction of epithelium, that it is, therefore, of a regenerative or, as we might more appropriately express it, of a "substitutive" character. On a former occasion we have adduced experimental facts which point to the conclusion that certain metabolic activities in the epithelium, which to some extent can be directed at will, determine the state of activity of the connective tissue. Those metabolic products which we called homoiotoxins depress the purely proliferative activity of the connective tissue cells and favor a quiescent stage, in which dense fibers are produced, while those metabolic products of the epithelium which are formed in the absence of homoiotoxins, stimulate the connective tissue cells in such a way that the fibroblasts remain intact and tend to multiply. Only fine fibrils are formed under those conditions. Especially noteworthy is the stimulating effect exerted by an active mammary gland. The active gland is surrounded by a cellular connective tissue, while the resting gland is surrounded by a dense fibrous tissue.

In our case we noticed a parallel development of gland ducts, chiefly resting, and of a surplus of dense fibrous tissue. Here again the relationship between epithelial and connective tissue structures is intimate. And, again, we may conclude that the connective tissue overgrowth is not the result of a primary destruction of epithelium, but of a stimulating effect exerted by the gland, and that under the peculiar metabolic conditions of the gland, the connective tissue becomes fibrous. We would again draw the conclusion that cirrhotic conditions may be due to a particular stimulating effect of a certain epithelial tissue on the surrounding connective tissue, under definite conditions of metabolism.

## SUMMARY.

Our observations are of interest in three directions:

1. They suggest a mode of origin of the fibroadenomata of the mammary gland in the rat. They show that two sets of factors may be responsible: (a) factors of a general character delaying the normal retrogression of several mammary glands, and (b) a local factor which determines that, instead of becoming necrotic and liquefying, as in the other glands, the tissue shall be preserved and even proliferate slightly.

2. They exemplify a peculiar type of retrogression of the mammary gland, in which great portions of tissue become necrotic en masse, and the epithelium of the gland ducts regenerates and separates the living from the dying tissue. This is a process similar to the casting off of dying deciduomata and placenta. In the end, the necrotic tissue becomes, at least in part, infiltrated with polynuclear leucocytes, and liquefies. Thus large cysts are formed.

3. They constitute a further argument in favor of the view that in certain cases cirrhotic conditions of an organ may not be of a substitute character, but rather the result of a direct stimulating effect exerted by the epithelium on the connective tissue, under conditions which favor the production of fibers by the fibroblasts rather than the integrity or multiplication of the connective tissue cells.

(1) Loeb, Leo: Further investigations in transplantation of tumors. Jour. Med. Research, 1902, N. S., III, 44.

Loeb, Leo and Fleisher, Moyer S.: Transplantation of benign tumors. Jour. Cancer Research, this issue.

## Plate I

Fig. 1. (Low magnification) shows a part of a cyst with papillary processes and necrotic masses. *a*, connective tissue of cyst wall, mostly lined by epithelium; *b*, three smaller cysts in the wall of the larger cyst. These smaller cysts are filled with dying tissue and leucocytes. *d, dI, dII,* places where the epithelium has not yet completely regenerated under the dying tissue and where the latter is in direct connection with the underlying connective tissue. *CI, CII, CIII,* necrotic material, the remnants of degenerated tissue, partly infiltrated by polynuclear leucocytes. In *CIII* the solution of the necrotic material has progressed farthest.

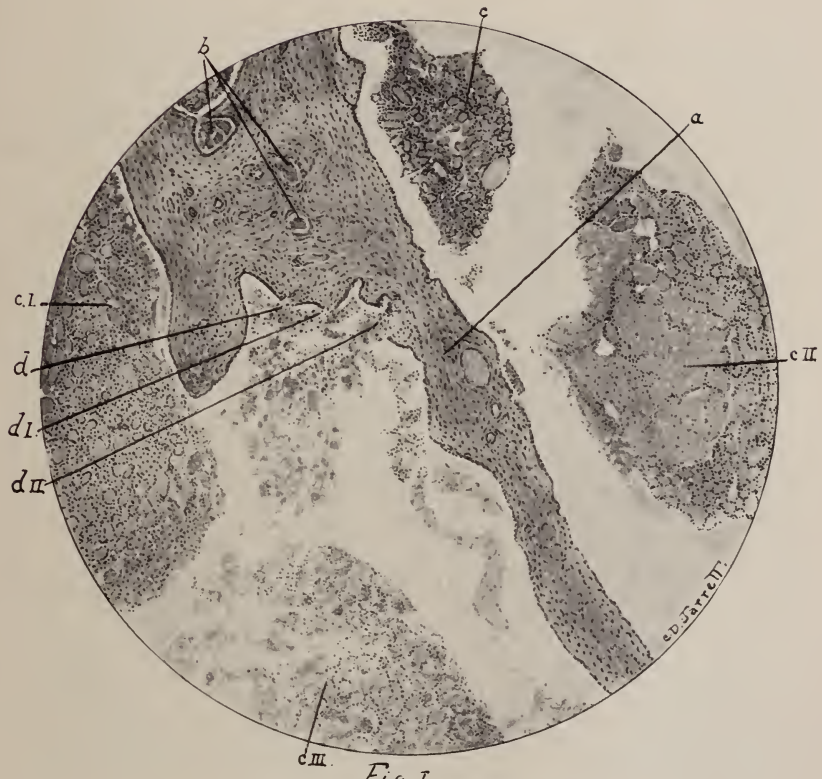


Fig. I.

## Plate II

Fig. 2. The part surrounding *d*, *dI* and *dII* of figure 1 in somewhat higher magnification. *e*, epithelial lining of cyst. *m*, dense connective tissue of cyst wall. At *d*, *dI* and *dII*, the epithelial covering is missing; here the regeneration has as yet been incomplete. The connective tissue cells swell and produce polygonal structures, *g*. The fibrils disappear between these swollen cells; the cells fuse to larger hyaline masses, *f*, and later to still larger masses, *h*. These become dissolved. *v* and *vI*, two capillaries included in the tissue which is in process of necrosis. *i*, polynuclear leucocytes which migrate through the cyst wall into the necrotic material.

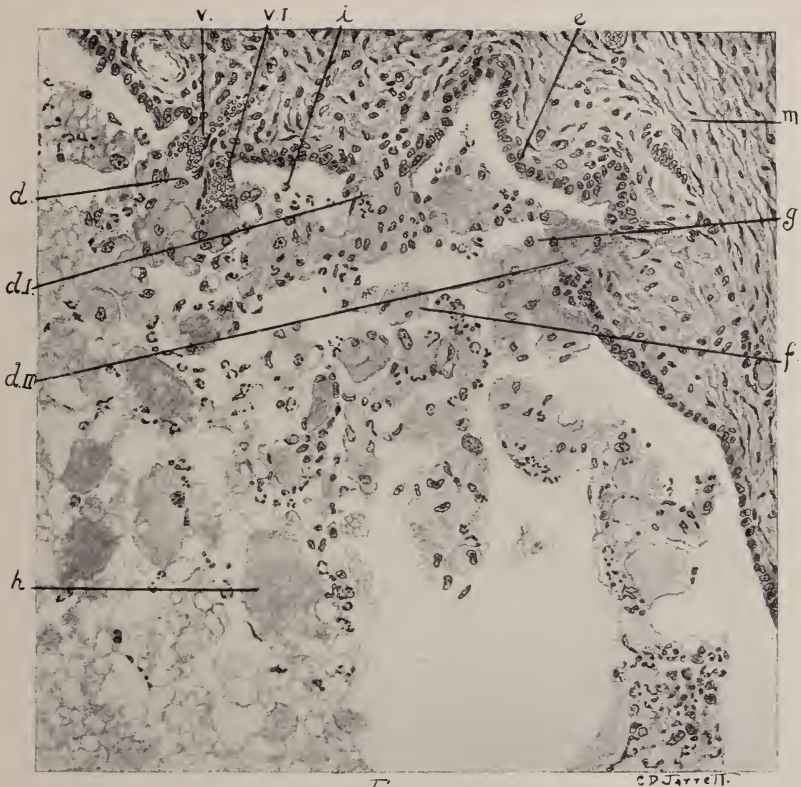


*Fig. II.*

# TRANSPLANTATION OF BENIGN TUMORS

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While the conditions under which carcinoma and sarcoma can be transplanted are on the whole fairly well known, thanks to a large number of contributions published in the last sixteen years, our knowledge concerning the growth and transmissibility of non-malignant tumors is as yet very restricted. An answer to the following problems would be of great theoretical interest. How far do benign tumors differ from cancer in conditions of experimental growth? How does their growth compare with that of normal tissues? Is it easier to bring about by experimental means the transformation of a benign into a malignant tumor than to effect a similar transformation of normal tissues? These questions are to a great extent still unanswered, owing to the limited number of investigations concerned with these problems. The present paper is a contribution to this field of study. It is the direct sequel of two papers on the growth of benign tumors previously published by one of the authors. At the outset it may be stated that while the results thus far obtained are of interest, much additional work will be required for the definite solution of these problems.

The first recorded experimental investigation into the growth of a non-malignant tumor was published by one of us in 1902, and was concerned with an adenofibroma of the mammary gland of a rat (1). After autotransplantation, the graft remained alive in toto but grew only during pregnancy; after homoiotransplantation, only very small peripheral areas remained alive for a time, and showed transitory regenerative growth.

The second publication (2) concerned an adenochondromyxofibroma of the mammary gland of a dog. Here again after autotransplantation the tissue remained alive almost completely, while it perished after homoiotransplantation.

H. Ribbert's experiment (3), which followed in 1910, confirmed our result. This investigator was able to transplant a fibroma of the dog into the same animal in which it had originated, but not into another dog. It began to grow in the first dog after a relatively long period of latency.

In this connection it will be of interest to refer to the behavior of normal tissues after serial regeneration. One of us found that it is possible to transplant serially, through a number of generations, normal epithelium of the guinea pig. Under these conditions an increase in the proliferative power of the epithelium is not observed, and after the typical period of regenerative growth the epithelium returns to its old equilibrium (4). C. V. Craster (5) subsequently reported similar experiments in which the skin of the rat was transplanted serially. No increase in virulence was observed. This author observed living cells as late as sixteen days after the first transplantation; he found no proliferation of the transplanted tissues. It is of interest to note that while Loeb in the main employed autotransplantation, in Craster's experiments the skin was serially transplanted into other individuals.

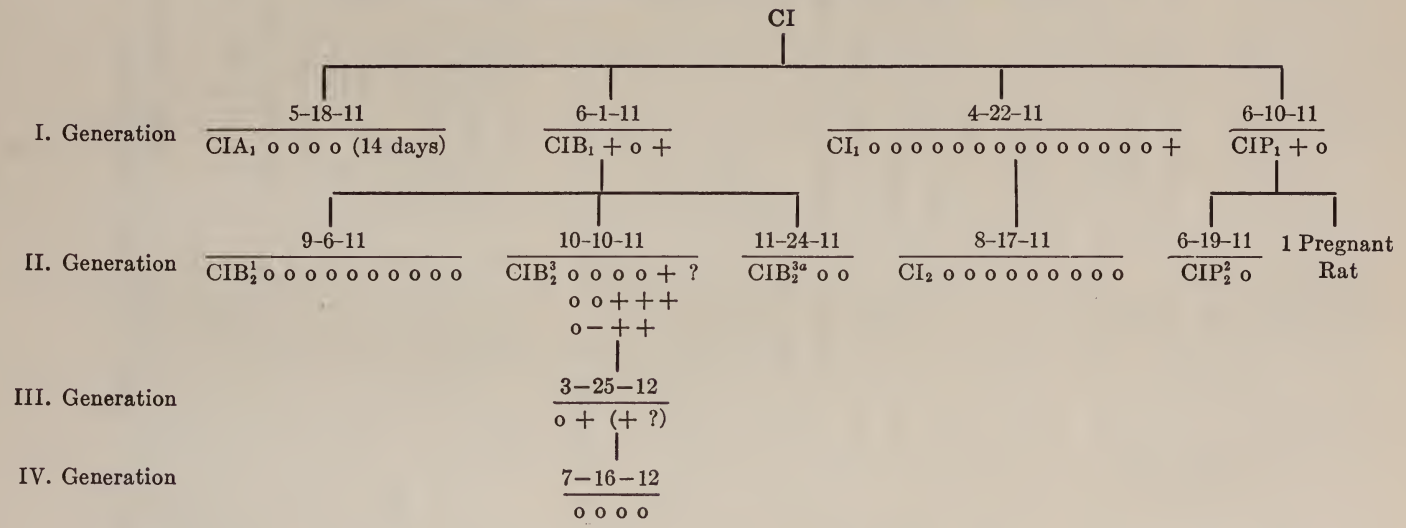
### FIRST TUMOR

(See chart)

A rat was received from Dr. J. W. Jobling, of Chicago, April 20, 1911. The animal had a tumor on the left side of the posterior part of the abdominal wall. The tumor was in the region of the posterior mammary gland.

On April 22, 1911, a piece of the tumor was removed and pieces about the size of half a pea were inoculated with a trocar into the right axilla of fifteen rats of different ages, females and males. On May 2, the inoculated pieces could be felt; eight days later (eighteen days after inoculation), they had become smaller or had disappeared. Four weeks after inoculation none

*Chart representing transplantation of the first tumor into four generations*



of the inoculated pieces was growing. However, between this latter time and the following August the inoculated piece in one of the rats began to enlarge and had reached a considerable size by August 17 (three and one half months after inoculation), on which date the animal was found dead. The tumor measured approximately 43 by 20 mm.

The tumor of the dead rat was removed and inoculated into nine rats on August 17, 1911. On September 6 (twenty days after inoculation) the transplanted pieces could still be felt, but on October 10, they had disappeared. On November 7, seven of the rats were still alive and on February 5, 1912, three. In none of them did a tumor develop.

On April 22, 1911, at the time of the first inoculation, a piece of the tumor was also transplanted subcutaneously into the rat in which the tumor had originated. This piece did not grow during a period of observation lasting thirty-nine days.

On May 18, 1911, another piece was removed from the original tumor and inoculated into four rats. One of these rats died nine days later; the other three fragments were removed fourteen days after inoculation. At this time the transplanted grafts had not noticeably changed in size. (Pieces $A_1$, $A_2$, $A_3$.) At the time of this last inoculation a piece of tumor was transplanted into the original tumor rat also. This likewise was removed two weeks later. It had not grown at the time of removal.

On June 1, 1911, a third piece of the original tumor was removed and was inoculated into two female rats and one male rat. Three weeks after inoculation, the grafts could no longer be felt through the skin, but on August 17 (two and one-half months after inoculation) the pieces in the two female rats were found to have grown to considerable size. At that time (August 17) one of these rats gave birth to a litter of four young. In the male rat no tumor developed during the period of observation, which lasted until October 10, four months and nine days after transplantation.

The first of the two tumors (B) which developed in the female rats, continued to grow from August 17, when it measured 27

by 27 mm., to September 6, a period of twenty days, at the end of which time it measured approximately 33 by 30 mm. On the latter date, a piece of this tumor was removed and was used for inoculation into a second generation of rats (eight of which were males and two females). A piece which had apparently not grown, was removed from a rat used in the last inoculation; this rat died five weeks after operation (October 9, 1911). The majority of the inoculated rats were still under observation two months after inoculation (November 7). On that date no tumor had developed.

The tumor which had grown in the second female rat of the last lot of the first generation ($B_3$), continued to grow definitely during the period from August 17 to September 6. On the latter date it measured approximately 25 by 25 mm. From then on, until October 10, no further increase in size was noticeable. On October 10, a part of the tumor was used to inoculate twelve rats. Two rats among this lot apparently showed a slight growth twenty days after inoculation, but they died soon afterwards. In one of the surviving rats ($C_1$), a growing nodule could be felt (January 18, 1912) more than three months after the inoculation; it continued to grow definitely until March 5, 1912. On that date a part of the tumor was removed and used for the inoculation of thirteen rats of the third generation; at the same time a piece was transplanted into the same rat ($C_1$). This rat ($C_1$) was observed until the death of the animal on July 10, 1912. On that date, neither the remaining part of the tumor nor the autotransplanted piece had grown. On February 6 (almost four months after inoculation), in another rat of this lot, a nodule appeared which measured approximately 11 by 8 mm. It grew slowly until March 15, at which time it measured 18 by 16 mm. The last observation was made on May 8. Among the fifteen rats of the third generation inoculated with tumor of rat $C_1$ on March 25, 1912, a few small nodules could be felt and were extirpated three months later (middle of June, 1912). Another graft, which had slightly enlarged, was taken out on October 4, 1912 (almost six and one-half months after inoculation), and the final nodule was removed

from the last rat of this lot on November 6, 1912 (seven and one-half months after inoculation). This latter piece had grown slightly and measured 8 by 4 mm.

On July 16, some pieces which could be felt were removed from five rats of the third generation and inoculated into four female rats of the fourth generation. One of these died six weeks later without tumor; of the remaining three animals, one had young in the latter part of August, 1912. It showed a very small nodule on October 4, more than two and one-half months after inoculation. At that date the animal was killed and the nodule extirpated. On November 6, 1912, one of the other rats also showed a very small nodule, which may have been the remnant of the inoculated piece. The fourth rat, which had young ones early in September, 1912, had no tumor at the time of the last examination, November 6, 1912.

The tumor $B_3$ of the first generation, from which a large piece had been removed on October 10, 1911, enlarged again and was inoculated into three rats on November 24, 1911, six weeks after it had been used for the first implantation; of these three rats of the second generation, one died soon after inoculation. Rat $B_3$ died on January 28, 1912. At the time of death only a small nodule was present. Another of the rats of the second generation died without a tumor on March 12, 1912, three and one-half months after inoculation. The third rat died with small nodules August 17, 1912, about eight and one-half to nine months after inoculation.

On June 10, 1911, the last piece was removed from the original tumor rat and inoculated into a pregnant rat and into another female rat which was apparently not pregnant. On June 18, eight days after inoculation, the pregnant rat littered. On the following day (June 19), the inoculated piece, which had begun to grow, probably under the influence of the pregnancy, and which measured 10 by 5 mm., was removed and inoculated into two female rats. No tumor growth resulted from this inoculation; two months later the inoculated pieces could no longer be felt.

MICROSCOPICAL EXAMINATION

*a. Original tumor*

1. Piece removed May 18, 1911 (no. 3). There are many mitoses in the epithelium, and some also in the connective tissue; but they are more frequent in the epithelium than in the connective tissue. The acini and ducts are surrounded by dense fibrous tissue with fairly numerous fibroblasts containing vesicular nuclei. In some places the acini are somewhat irregularly arranged, and there is a great deal of gland tissue which is rather diffusely distributed without the formation of definite lobules. Some of the ducts are somewhat dilated.

2. Piece removed June 1, 1911 (no. 5), used for inoculation. There are no mitoses visible either in the epithelium or the connective tissue; the lobules filled equally throughout with acini. The latter are surrounded by a dense fibrous connective tissue. These lobules are separated from each other by collections of connective tissue cells which do not contain epithelial structures. Ducts with small lumina are numerous. There is no irregularity in growth, and no dilatation of the ducts. Inoculation with these pieces led to the development of tumors in several of the rats, although no mitoses were present in the piece examined microscopically.

*b. Piece autotransplanted into original tumor rat*

This remained fourteen days in the original tumor rat—May 18 to June 1, 1911. The central parts are hyaline and necrotic; the peripheral parts contain cellular connective tissue. Toward the center, connective tissue cells as well as epithelium decrease around the ducts, the connective tissue becomes quite hyaline and compresses the ducts. In some areas the epithelium is lost and the connective tissue almost lost. There are probably a few mitoses in the connective tissue, but this is not certain. In this piece, the ducts are regularly branching and are very numerous.

$A_2$ (no. 1) inoculated May 18, 1911, and removed fourteen

days later. In some places the connective tissue is very rich in fibroblasts, at others it is clear and hyaline. The tumor consists of lobules, in the centers of which there is a system of glandular ducts surrounded by connective tissue. Where the connective tissue is very hyaline, the ducts are often dilated. There are a number of mitoses, and some amitotic divisions in the cellular connective tissue and also in the ducts, but no mitotic figures in the neighboring glandular epithelium.

$A_3$ (no. 6), fourteen days after transplantation. In the peripheral areas the connective tissue is well preserved; nearer the center, there is some hyaline tissue with a few connective tissue cells and gland ducts diminishing in number. Arrangement in lobules which are somewhat irregularly dilated, and surrounded by connective tissue which tends to become hyaline. The interstices between the lobules are filled by cellular connective tissue strands, which may be invasions from the host's tissue. In the peripheral parts an unusually large number of mitoses is found in the epithelium and in the connective tissue. In the latter the number is even larger than in the epithelium. The interstices contain, also, collections of small round cells, especially where the neighboring tissue becomes necrotic. They penetrate from here into the adjacent glandular structure.

$A_1$, May 18 to June 1, fourteen days after transplantation. Nodules of hyaline connective tissue some of which contain ducts. There is much round cell infiltration. The ducts are rather shrunken; on the whole there is very little epithelium left, and some of the epithelial cells are desquamated. There were pneumonic areas in the lung.

Piece (no. 4) transplanted into a pregnant rat June 1, 1911, removed on June 19, one day after delivery. The periphery is alive, but toward the center the graft is necrotic. Numerous mitoses occur in the epithelium; there are some mitotic figures in the connective tissue also, but here they are less numerous. The acini are surrounded by dense hyaline connective tissue containing some vesicular nuclei. Some of the ducts are dilated and irregular.

Piece inoculated April 22, 1911, removed May 10, 1911,

(eighteen days). In the center there is dense hyaline connective tissue; at the periphery, glands surrounded by cellular connective tissue. The tissue is shrunken, without any sign of proliferation.

$B_3$ (no. 11), June 1, 1911 to October 10, 1911. Examined four months and nine days after transplantation. The graft had been of considerable size two and one-half months after inoculation. It is composed of mammary acini and ducts, concentrically surrounded by dense hyaline connective tissue which is not very rich in cells. Some of the gland cells are vacuolated, and a few small mononuclear cells are occasionally found in the ducts. No mitoses are visible; there is some colloid in the acini. At some places the connective tissue is almost, or quite necrotic. In certain areas the structure is fairly typical, while others contain only isolated ducts without the typical ramifications.

$B_3$ (no. 12). The same tumor, after it had been used for two transplantations and only a small nodule had been left. Piece taken out after the death of the animal, June 1, 1911 to January 28, 1912 (seven months, twenty-one days). This fragment has the same structure as the first; the ducts and acini are surrounded by dense fibrous connective tissue. There are some fibroblasts in the connective tissue. Apparently no growth had taken place.

*Pieces of the second generation*

1. No. 13, rat inoculated with piece of tumor $B_1$ September 6, 1911, died October 9, 1911 (thirty-three days after inoculation). The specimen consists of hyaline connective tissue containing fibroblasts and a few blood-vessels, but no epithelial elements. No growth had taken place.

2. No. 14, a male mouse, October 10, 1911 to July 10, 1912, was found dead after nine months. The tumor had grown very slowly. Postmortem changes are present; the nuclei are shrunken, and the tumor consists of necrotic material and hyaline connective tissue containing some glandular structures. Concretions are to be found in some of the ducts.

3. No. 15, autotransplanted into previous rat March 25.

On July 10, 1912, the animal was found dead, eight months and sixteen days after inoculation. This piece, which corresponds to the third generation, consists mostly of necrotic dense fibrous tissue without nuclei. Postmortem changes had taken place. In one area the fibrous tissue contains well preserved acini, surrounded by fibroblasts; here the connective tissue is more cellular.

4. No. 16, October 10, 1911 to August 20, 1912 (ten months, ten days). A very small nodule, mostly necrotic; some of the lobules contain shrunken desquamated epithelium (possibly due to postmortem changes). The ducts and acini, where present, are surrounded by connective tissue containing nuclei, and in some of them concretions are to be found. Where the epithelium is lacking, the connective tissue is often necrotic, and the greater part of the tumor is entirely devoid of cells. In one epithelial cell there appears to be a mitotic figure. Some dilated ducts are present.

5. No. 17, November 24, 1911 to August 17, 1912 (eight months, twenty-four days). The animal was found dead. There are vestiges of acini in the dense hyaline connective tissue, but the fragment was necrotic, apparently as a result of postmortem changes.

*Pieces of the third generation.*

1. No. 21, March 25, 1912 to November 6, 1912 (seven months, eleven days). The small tumor, removed during the life of the animal, contains hyaline connective tissue arranged concentrically around ducts or acini often devoid of their epithelium. In some areas there are no nuclei in the connective tissue. Preservation of the connective tissue and the epithelium has run a parallel course. In some places all the ducts and acini in a lobule are preserved. Some of the acini are dilated, and at such places more fibroblasts are present in the connective tissue. The epithelium consists of relatively large cuboidal cells with nuclei rich in chromatin, a number of which are in mitosis. Where the epithelium is undergoing mitosis, the connective tissue is not so densely hyaline, but rarified, and connective

tissue cells are present in larger numbers; a little further away from such areas, however, the connective tissue surrounding the ducts is again densely hyaline. In the large ducts and also in the acini there is some colloid material. Around isolated ducts, the connective tissue is dense and contains fewer fibroblasts. Cyst-like dilatations of ducts are also present, directly surrounded by cellular connective tissue. Occasional mitoses are to be found in the connective tissue.

2. No. 20, March 25, 1912 to October 4, 1912 (six months, nine days). A small nodule which had apparently grown somewhat, composed of dense hyaline fibrous tissue, mostly necrotic, with a few nuclei, and without epithelial structures. Some of the cells in the connective tissue may have grown in from the outside.

3. No. 19, March 25, 1912 to June 17, 1912 (two months, twenty-three days). A very small necrotic nodule, with hyaline connective tissue. In the centers of areas of concentric hyaline connective tissue lie some shrunken ducts. It is impossible to state to what extent postmortem changes are responsible for these appearances.

4. No. 18 March 25, 1912 to June 13, 1912 (two months, nineteen days). A fragment consisting of connective tissue and shrunken vessels which is almost entirely necrotic.

*Piece of the fourth generation*

No. 23, inoculated July 16, 1912 to October 4, 1912 (two months, eighteen days). A small inactive nodule of the third generation which had been used for transplantation. It consists of hyaline connective tissue, mostly necrotic, and without nuclei; in certain areas at the periphery some nuclei are to be found, which, however, had probably immigrated from the surrounding tissue of the host.

FOURTH TUMOR

A rat with a large tumor under the skin covering the sternum, was received from a Philadelphia dealer on October 4, 1911. On October 10, three rats obtained from Philadelphia and three

from Chicago were inoculated, and a piece of the tumor was transplanted into the original tumor rat. The original tumor rat died on October 19. The autotransplanted fragment was not yet very firmly fixed. On November 7, four weeks after inoculation, no tumors had appeared in the inoculated animals, and soon afterwards the majority of the rats died. In the only one that survived longer, no tumor had developed when it died on March 5, 1912, almost three months after inoculation. A small nodule was, however, still present at the place of inoculation.

The microscopical examination of a piece of the original tumor, taken out some time after the death of the animal, showed merely dense fibrous tissue with a necrotic center.

The piece that had been transplanted into the original tumor animal consists also of fibrous tissue which is mostly necrotic, but in some peripheral areas numerous connective tissue cells are present. In this, as well as in the original tumor that had not been transplanted, some of the peripheral cells may possibly have immigrated into the tumor from the peripheral tissue of the host. A nodule removed from the rat which was found dead on March 5, 1912, has the same structure; nowhere is there any indication of growth.

### SECOND TUMOR (GRANBY)

The rat bearing this growth was received in the spring of 1913 from Granby, Mass. The tumor was situated in the right axilla toward the back. On May 6, 1913, a fragment was removed from the tumor, which was very firm; two grafts were autotransplanted into the dorsal subcutaneous tissue of the tumor rat, and six normal rats were inoculated. None of the pieces grew.

On July 8, two months after the inoculation, the homoiotransplanted grafts, which had apparently not grown, were removed, as well as a piece (no. 406), which had been transplanted into the tumor rat. At the same time another was removed from the original tumor and fragments were autotransplanted at three different sites in the original tumor rat. Six normal

rats were also inoculated. Sixteen days later (July 22, 1913), the transplanted pieces were removed from two normal rats (no. 405), and one autotransplanted piece from the tumor rat. On August 20 (one month, twelve days after the second inoculation), the grafts were removed from two of the inoculated rats (no. 407), and one autotransplanted piece from the tumor rat (no. 403) was extirpated. The tumor rat died on the day following the last operation. The original tumor, from which pieces had been removed on two occasions, had grown noticeably. After the death of the rat it was dissected out and found to consist of two parts, a firm white portion (no. 401) from which the pieces had been previously removed, and a larger soft, pink mass (no. 402). It is not improbable that the latter represents that portion of the tumor which had grown since the operation, and perhaps as a result of the stimulating effect of the operation. On sectioning, the soft part shows a distinctly lobulated arrangement, and there was a sharp line of demarcation between the hard and the soft regions. The lungs presented a smooth appearance, white and pink areas alternating with each other. The remaining organs were normal. Six rats were inoculated with grafts from the soft part, but no growth took place.

The microscopical examination showed the following:

1. The hard part of the original tumor, removed at the time of the animal's death, consists of dense hyaline fibrous tissue with slender nuclei, arranged in bundles, and containing compressed ducts lined with low epithelium. Some of these ducts seem to have atrophied under the influence of the pressure. The connective tissue, which at various places contains fat cells, is concentrically arranged around the compressed ducts.

2. The soft part of the tumor is very much richer in ducts than the firm portion; their lumina are larger and their epithelium higher, but mitoses are absent. Though the connective tissue is, on the whole, hyaline, it contains a larger number of nuclei. At some places fibroblasts are so numerous that the absorption of hyaline connective tissue has been brought about. Here again the connective tissue is usually arranged around the ducts as centers of lobules. Some ducts are much dilated; in other

regions they are the seat of irregular outgrowths. Large vessels occur occasionally in the tumor.

3. The first autotransplant (No. 406), May 6 to July 8, removed after two months, has the same structure as the original tumor. At the periphery, some of the ducts are preserved, and the hyaline connective tissue is concentrically arranged around them. In some of the ducts the epithelium is higher than in others and some ducts are surrounded by more connective tissue cells than others. Mitoses are nowhere visible. From the peripheral tissue of the host, connective tissue cells have immigrated into the tumor tissue and separated the individual lobules. In the center of the piece, ducts as well as connective tissue cells have disappeared.

4. An autotransplant from the second inoculation (no. 404), July 8 to July 22, removed after two weeks, has on the whole a similar structure, but there is evidence of infection at one point, and this may have injured the tissue to some extent. No mitoses are seen. The connective tissue is dense and hyaline, and contains nuclei, and narrow compressed ducts, either without a lumen or with a very small one. At the periphery, connective tissue from the host is growing into the piece.

5. The corresponding homoiotransplant (no. 405), removed after two weeks, has, at the periphery, well preserved ducts, some of which contain mitotic figures. The connective tissue around them is poor in nuclei, or the latter may be entirely missing. There is some connective tissue from the host growing between the hyaline tumor lobules. In the center of the graft the ducts are degenerating. The epithelial cells swell and become vacuolated and the nuclei become paler. In the center of the piece connective tissue has also been lost. The proliferation in the glandular structure, which we find in this case, may have been the result of wound stimulus.

6. An autotransplant (no. 403) from the second inoculation, removed after six weeks, contains hyaline connective tissue with relatively few nuclei, and some narrow compressed ducts in the center of it. In some places, the connective tissue is absent altogether. Mitoses are nowhere visible. In the center

the ducts seem to be degenerated, but some ducts are preserved even well toward the center.

7. A corresponding homoiotransplant (no. 407) of the same period contains hyaline connective tissue with very few connective tissue cells, and small narrow ducts which usually have vesicular epithelial cells with deformed nuclei. In some areas, the ducts are somewhat better preserved. The nuclei of the connective tissue cells are to a great extent lost.

In this case we have to deal with the same kind of adenofibroma of the mammary gland as in the first animal. Although two weeks after transplantation there was some proliferation of the ducts, no definite growth took place in them in the homoiotransplanted pieces as a whole. The ducts determine, as is usual in these tumors, the structure of the whole neoplasm. In both autotransplanted and homoiotransplanted pieces central necrosis is present, but the area which remains alive directly after transplantation is in these tumors much larger than in the case of either mouse carcinoma or rat sarcoma. The fibrous connective tissue is apparently more resistant, and also preserves the enclosed ducts to some extent. While no proliferation took place after transplantation, a considerable part of the tissue, at least, was preserved.

### THIRD TUMOR[1]

The rat was probably about two years old when received. In the right groin of the animal there was a tumor of lobulated structure, consisting of glandular ducts surrounded by fibrous and largely hyaline connective tissue. In some of the ducts the epithelial cells are somewhat deformed and vacuolated, while in others they are well preserved. A gland duct with connective tissue concentrically arranged around it forms lobules. Between the latter there are ramifying strands of connective tissue cells without ducts, which have probably pushed in between the lobules from the surrounding normal tissue.

[1] This rat was received from the Wistar Institute in Philadelphia through the courtesy of Dr. H. H. Donaldson.

On October 16, 1914, pieces of the tumor were transplanted into the tumor rat, into ten rats belonging to the same family as the tumor rat, and into several non-related rats. Five days after the operation the tumor rat died. The inoculated animals were observed during the remainder of their lives, some during a period of almost six months, at the expiration of which they all had died. No tumor developed in any of them. This case constituted another instance of an adenofibroma of a rat, in which inoculation was unsuccessful.

### FIFTH TUMOR

A second rat with a tumor was obtained from Philadelphia on October 4, 1911. The tumor was situated on the back, in the region of the left fore leg. The rat was a female. On October 10, a part of the tumor was removed and six rats were inoculated; another piece was transplanted into the tumor rat. On February 6 (almost four months after the inoculation) three rats were still alive. No tumor had developed; the remaining three animals had died without tumors. On October 20 (ten days after the inoculation), the autotransplanted piece had grown a little, and then remained for a short time apparently stationary. On November 15, however (thirty-six days after inoculation), it had become decidedly larger, measuring 10 by 12 mm. It continued to enlarge until on November 24 (forty-three days after inoculation) it measured 15 by 17 mm. The following day a part of the autotransplanted tumor was removed and a second piece transplanted into the rat with the original tumor, which had in the meantime showed active growth. At the same time three other rats were inoculated. On March 7, 1912 (almost three and one-half months after inoculation) no tumor had developed in the two rats which had survived up to that time, and one lived until May 8 without developing a tumor. Neither did the autotransplanted piece, inoculated on November 25, show growth at the time of animal's death on February 24, 1912, three months after inoculation.

After the second transplantation, on November 24, the original tumor decreased somewhat in size during the following two and

one-half months, and on February 6 measured 15 by 15 mm. The animal became thin and emaciated, and the first autotransplanted piece also diminished in size.

On February 13, 1912, the greater part of the original tumor was removed and pieces were inoculated into four rats. On the day following the last operation the tumor rat died. No growth developed in the inoculated rats, six of which were observed until June 25, 1912 (four months, twelve days).

The microscopical examination of the first autotransplanted piece, taken out forty-five days after inoculation, showed a very cellular, myxoid connective tissue with rather large vesicular nuclei; the tumor was traversed by capillary blood-vessels. A number of mitoses were present, some of which were irregular. At other places the tumor was necrotic. A few polynuclear leucocytes were seen in the tissue. The tumor consisted entirely of connective tissue, resembling the fourth tumor; it was not an adenofibroma, as were the first three tumors. In this case we are perhaps no longer dealing with an entirely benign tumor. It is of interest to note that an autotransplanted piece grew, while the homoiotransplanted piece did not grow, an observation in agreement with our previous results. It is furthermore of interest that when the general health of the original tumor rat suffered, the original tumor and the first autotransplant decreased in size and a second piece autotransplanted during this period did not grow.

### SIXTH TUMOR

In this connection it might be of interest to mention a rat which was received from Granby, Massachusetts, in June, 1912. This animal had a large mammary tumor consisting mainly of mammary gland tissue, in which the cells are filled with vacuoles, probably containing fat, and arranged in the form of solid alveoli. At other places there are acini with wide lumina and cells showing a homogeneous protoplasm, or filled with vacuoles. The alveoli and acini are arranged in lobules which are separated by strands of dense fibrous tissue. We are evidently in this

case dealing with a hypertrophic secreting gland which has failed to undergo involution.

## CONCLUSIONS

1. We may conclude from these studies that it is possible to transplant benign tumors, specifically the adenofibroma of the mammary gland of rats, through several generations. We succeeded in demonstrating growth through three generations, not only by following the increase in size, but also by demonstrating proliferation microscopically many months after inoculation. It is quite possible that if we had been able to carry out the experiments on a large scale, the propagation could have been continued into further generations. It is of interest in this connection to recall the fact that in the case of normal tissue (skin) one of us was able to obtain successful "serial transplantations," but the transplanted normal tissue very soon regained its old equilibrium and did not acquire a long continued expansive growth, in contradistinction to benign tumors, which may show a long continued growth after transplantation. Thus each tissue after transplantation retains, on the whole, the characteristics which it possessed before transplantation.

2. If we compare the growth in the different generations, we notice an apparent decrease in the growth energy in each successive generation. The latent period increases, and the subsequent microscopically determined growth becomes slower in the second and third generations. While we must take into consideration the fact that in the first generation also variations occur in the number of growing tumors in the different sets of inoculations, still the gross difference between the various generations is sufficiently pronounced to give to this conclusion a considerable degree of probability. In the first generation we find several large tumors within a period of time varying between two and four months; in the second, the largest tumor obtained is considerably smaller five months after transplantation than the tumors of the first generation after two and four months. In the third generation we find a growing nodule with mitoses

seven months after transplantation, but it is still small even at this time.

We may then conclude that in all probability benign and malignant tumors differ after transplantation in a definite way. As Loeb has found, sarcoma as well as carcinoma generally show upon transplantation a definite increase in growth energy, which is not dependent on a selection of the most virulent cells, as Ehrlich maintained, but upon a direct stimulation of the tumor cells by the conditions associated with transplantation. The curve of this increase differs in different tumors. In benign growths, on the other hand, this increase in growth energy seems to be absent; on the contrary, there is a gradual decrease, and they seem, therefore, to stand in this respect somewhere between normal tissues and cancers. It would be of great interest to attempt an increase in the growth energy of originally benign tumors also.

3. In the case of our first tumor the period of latency is relatively great, comprising several weeks even in the most favorable transplants. Microscopically, however, we may find numerous mitotic figures nine to fourteen days after transplantation. While it is possible that in some cases this marked proliferation was essentially regenerative in character, it is probable that such regenerative growth in the case of benign tumors exceeds that of normal tissue at corresponding periods after transplantation. There is therefore added to the mere regenerative external stimulus a condition within the tumor that leads to cell proliferation.

4. There is a certain variability in the results of transplantation in the first as well as in the succeeding generations. Especially noticeable is the fact that the first inoculation with pieces of the first original tumor (April 22, 1911), did not lead to as good a result as the later inoculations. It is possible that the stimulating effect of the first extirpation of pieces from the original tumor made the latter more effective in the following inoculations, just as the extirpation may have had a stimulating effect on the proliferation in the original tumor. We also notice that the piece inoculated into a pregnant rat on June 10, 1911, grew definitely within a short period of time. This is an observation which

accords with the earlier one of Loeb on the stimulating effect of pregnancy on the growth of mammary adenofibroma. It is of interest that in the benign mammary tumor the same substances which regulate the growth of the normal gland should be still active. The chemical constitution of the adenofibroma shows, therefore, the same specificity as the normal tissue from which it is derived. While chemical factors evidently play a certain rôle in the growth of the adenofibroma after transplantation, such favorable substances are not limited to female rats; the tumor growing most actively in the second generation was observed in a male.

5. At various periods after transplantation mitotic figures can be found, which are occasionally more numerous in the connective tissue than in the epithelial structures. We may therefore conclude that the increase in proliferative power found in the adenofibroma affects both the epithelial and the connective tissue structures, perhaps independently of one another. In this respect we should have, therefore, not only an adenomatous, but also a fibromatous tumor, and the designation "adenofibroma" would be appropriate. The epithelial components, on the other hand, are the determining factors as far as the structure of the tumor is concerned; in the center of the lobules we find glandular ducts or acini around which the connective tissue is arranged in a more or less concentric fashion. While, therefore, epithelium and connective tissue show an independent tendency to cell proliferation in the case of our tumor, a correlation between the two tissues still exists.

6. Tumors II and III, which also were adenofibromata of the mammary gland of the rat, did not yield growing tumors. Neither the auto- nor the homoiotransplanted pieces grew, but a considerable part of the transplanted pieces remained alive. We can not, of course, exclude the possibility that if a still greater number of rats had been inoculated and if the observation had extended over a still longer period, some positive results might have been obtained. But at present it appears at least probable that exactly as with malignant tumors, there is also among benign tumors a difference in transplantability; some appear to

be more readily transplantable than others. As Loeb stated in the report of his first transplantation of rat sarcoma, a similar structure in tumors may be associated with differences in biological characteristics.

7. Those pieces of adenofibromata which do not grow after transplantation seem at least to remain alive for a considerable period of time, and this applies to the auto-, as well as to the homoiotransplanted pieces. In those pieces that do not show any definite growth some mitoses may be found within the first few weeks after transplantation, probably in response to the regenerative stimulus of the experiment.

It is of interest that the area that remains alive after transplantation of these adenofibromata of the rat is considerably larger than the area that survives after transplantation of carcinomata or sarcomata in the rat or mouse. But in all cases the central parts of the tumors become necrotic even after autotransplantation. This confirms the previous observations of Loeb in the case of transplantation of benign tumors.

While, however, in the former transplantations of adenomata or fibrochondroadenomata of the mammary gland in the rat and dog, a considerable part or all of the transplanted tissue remained alive only in the original tumor animal, in contradistinction to other individuals of the same species where the pieces soon became necrotic in toto, in the present series of experiments we find that even in certain other individuals a considerable part of the peripheral tumor tissue remains alive. But while in our earlier work in the rat even the central necrosis was absent after autotransplantation, we find in this third series of experiments indications of central necrosis in many transplanted pieces.

The increase in the area of living tissue after auto-, or, in certain cases even after homoiotransplantation of tissues, may be due in part to the greater resistance of the fibromatous tissue which shields also the included glandular structures. In addition, less actively growing tissues are less sensitive to injurious influences, and actively growing cancer cells are more sensitive—they do not survive as readily as cells of benign tumors. Furthermore, the preservation of blood-vessels in the transplanted tissue

which join with the new capillaries formed by the host, may insure in the adenofibroma a more rapid restoration of circulation than in the carcinoma, where many or all of the irregular, less well-formed blood-vessels perish soon after transplantation.

8. Two purely fibromatous tumors, the second of which approached a sarcomatous nature, were not successfully transplanted into other rats. In the case of the second of these tumors it is interesting to note that while the homoiotransplanted pieces did not grow, the autotransplanted piece grew and showed mitoses six and one-half weeks after transplantation. This is an occurrence in accordance with the first observations of Loeb, who showed that very often tumors grow or remain alive after autotransplantation, while they die after homoiotransplantation.

It is furthermore of interest to note that the fate of both the original tumor and the autotransplanted pieces may depend upon the general health of the animal. Just as in the case of homoiotransplanted pieces the general state of health is one of the factors that determine the rate of growth of the inoculated pieces, so we find that in the case of the second fibromatous tumor the general condition of the animal determined a decrease in size of the autochthonous tumor and at the same time prevented the growth of the second lot of autotransplanted pieces.

9. If we compare the fate of the various non-malignant tumors after transplantation, we find some interesting variations. In the first mammary adenoma of the rat, on which one of us reported in 1902, the autotransplanted piece remained alive in whole, or at least in the greater part, while after homoiotransplantation only a small peripheral part remained alive and showed temporary regenerative growth. In the adenochondrofibroma of the dog, the pieces remained alive almost in toto after autotransplantation, while after homoiotransplantation they became necrotic. No growth took place.

In a similar manner we found growth in one Philadelphia myxofibroma only in an autotransplanted piece, not in the homoiotransplanted pieces. In the Chicago adenofibroma large parts remained alive and grew even after homoiotransplantation;

and in the Granby adenofibroma parts of the homoiotransplanted tumor apparently remained alive for some time, although we do not find growth in any of the pieces. In two cases we find an influence of the ovarian internal secretion on the growth of the adenofibroma of the rat; in our previously reported case in the autotransplanted piece, and in the Chicago tumor in the homoiotransplanted piece.

It is apparent that there are variations in the resistance to homoiotoxins as well as in the growth energy in the case of different non-malignant tumors.

10. It is of interest that the transplantation of adenomafibroma of the mammary gland into pregnant rats seems to favor its growth during the period of pregnancy, much as the growth of the transplanted normal mammary gland is favored through pregnancy. If, on the other hand, carcinoma of the mammary gland of the mouse is transplanted into other pregnant mice, it usually does not grow. Normal embryonal tissue behaves in a manner similar to carcinoma in the mouse, but apparently not in the rat.

We are in these cases evidently dealing with an equation containing four variables—namely, (a) the specific affinity of the transplanted tissue for a certain growth substance given off by the ovaries. This affinity is greatest in the case of normal mammary gland tissue and of adenofibroma of the mammary gland; it is less marked in the carcinoma of the mammary gland and lacking in the ordinary embryonal tissue. (b) A factor injurious to tissue growth operating in pregnancy. This may be either a directly injurious substance or a shortage of ordinary food-stuffs due to the growth of the embryo. There are certain facts which suggest the first alternative rather than the latter. (c) Homoiotoxins seem to strengthen the second injurious factor, while their absence seems to favor the first aiding factor. (d) There seem to be variations in the strength of one or several of these variable factors in various species. In the mouse the injurious factors seem to be relatively stronger than in the rat. The manner in which these variables combine determines the end result.

11. There are certain structural abnormalities in the growth of the adenofibroma which distinguish its growth from that of the normal mammary gland. On the whole they are slight; they correspond to a slightly increased growth tendency under abnormal conditions. It was occasionally found in pieces of the original tumor as well as in transplanted pieces. We observed irregularities in the structure of the acini, due either to ingrowth of some cells or to the disappearance of the walls separating neighboring acini.

This increase in growth energy implies a somewhat greater intensity of growth processes, even in the absence of specific growth stimuli, and also the failure to attain perfect retrogression at periods of rest, which characterizes the normal gland. Here again slight morphological peculiarities go hand in hand with slight biological differences and the former are probably the expression of the latter. However, biological as well as morphological peculiarities are such as are associated with benign tumors rather than with cancerous growths. We must, however, remember that sharp demarcations between these conditions do not exist, that the demarcation is within certain limits arbitrary. In our case the deviation from the normal is as yet relatively so slight that our tumor cannot be classed among the carcinomata.

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# PLATES

## PLATE I

FIG. 1. ORIGINAL TUMOR

*c*, dense fibrous tissue; *d*, a somewhat irregularly formed gland. *a*, part of the tumor which contains a relatively small number of glands. *b*, part of the tumor rich in glandular structures.

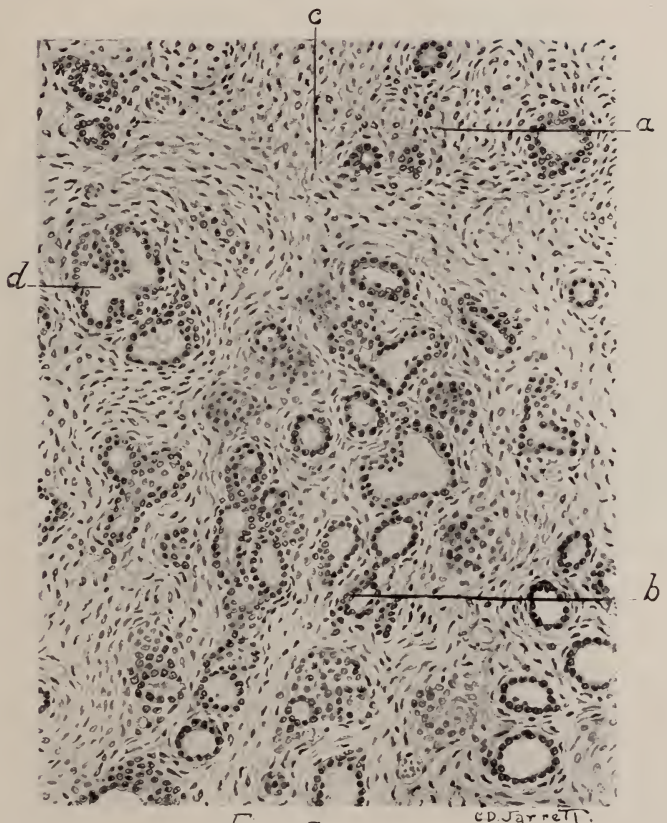


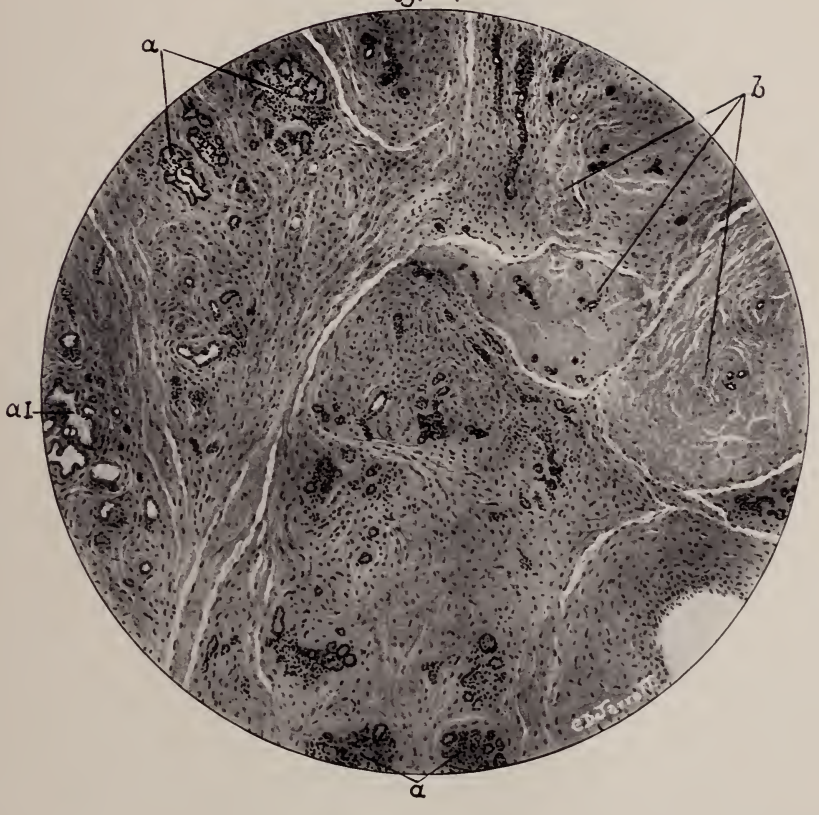

Fig. I.

## PLATE II

FIG. 2. TRANSPLANTED TUMOR, 3D GENERATION

*a* and $a_1$, glandular structures in the periphery of the transplanted piece. $a^1$, collections of glandular structures in which are situated formations which are reproduced in higher power in figures 3 and 4. *b*, area toward the center of the transplanted piece; here the number of living glandular structures decreases and necrosis begins.

Fig. II.



## PLATE III

FIG. 3. IRREGULAR CONVOLUTION OF GLANDS NEAR *a*I OF FIG. 2

*c*, surrounding connective tissue between the glands; in the center of the drawing between the acini the connective tissue is becoming dissolved; *a*, irregular gland convolutions; *m*, mitosis in a gland cell.

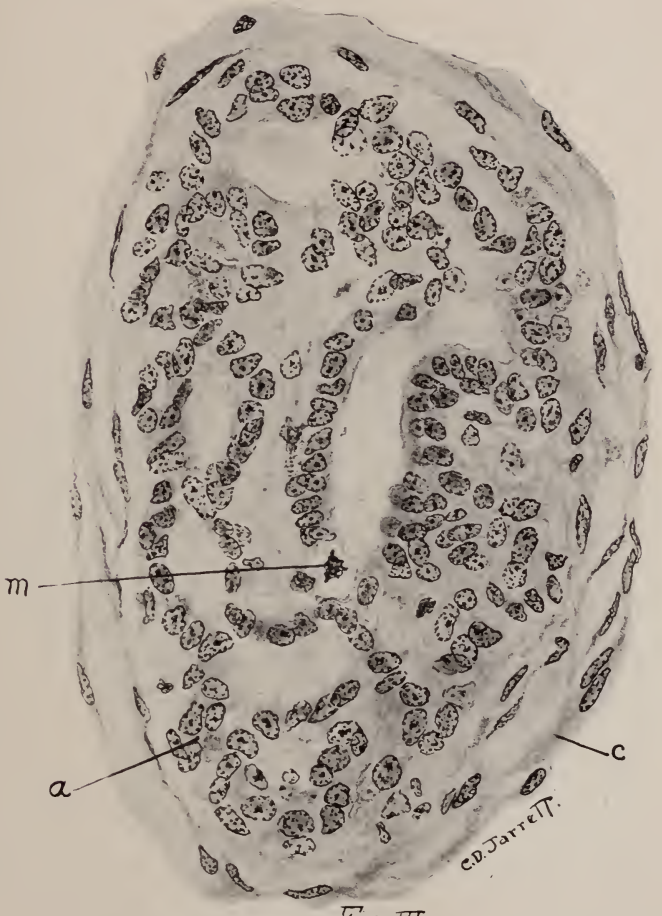



Fig. III.

## PLATE IV

FIG 4. IRREGULAR GLAND STRUCTURES FROM THE SAME PLACE AS FIG. 3

*b*, surrounding connective tissue; *a*, epithelial structures filling out a considerable part of the cavity; *c*, 2 mitoses. *l*, immigrated lymphocyte.

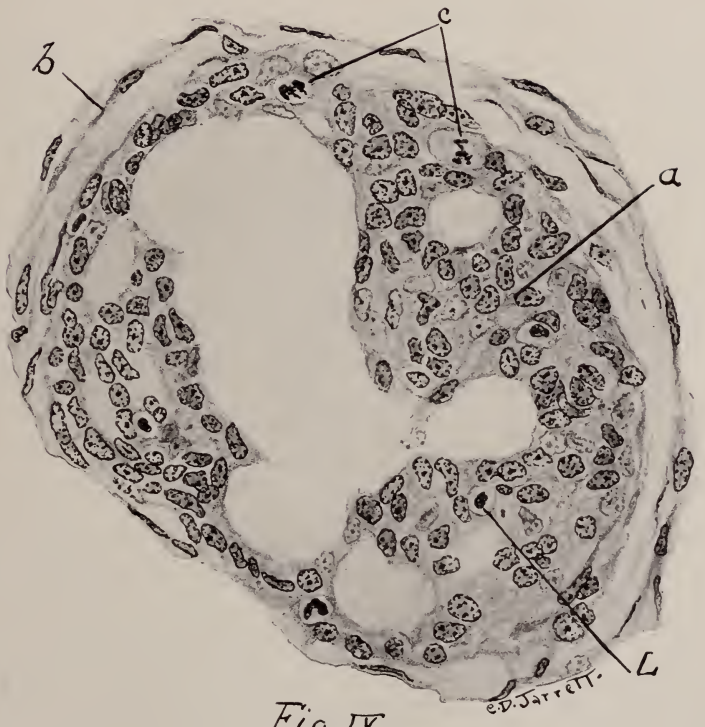



Fig. IV.

# PRIMARY CARCINOMA OF THE URETER

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The first case of primary carcinoma of the ureter appearing in the literature was reported in 1878. However, no review of the literature on this topic was made until 1909, when Zironi (19) reviewed 5 cases. Richter (13), in the same year, reviewed 11 cases including all of those in Zironi's list. Chevassu and Mock (3) found 12 cases. Chiari (4), in 1914, succeeded in gleaning 17 cases from the literature, a few of which, however, cannot be classed under the list of malignant tumors. Spiess (15), in 1915, made the most thorough review to date, including the benign and malignant growths of both the ureter and the kidney.[1] He lacked access to a few papers but indicated them.

With the reporting of a case of primary carcinoma of the ureter at autopsy, it was decided to review the cases on record of cancer limited to this particular structure and include them in this report, thus adding four more cases to the literature. The cases follow in chronological order.

Case I. Wising and Blix (18). *History and symptoms.* Age 41 years, woman. Several months previous to observation, while in a crowd, was pushed against a wall with pressure on abdomen. Since then pain in the right lumbar region. Later patient became aware of a lump in the abdomen.

*Examination.* When first seen a tumor was palpable in the right abdomen, elongated but smooth. Two months later there was an appreciable increase in size. A few small nodules present anterior to the tumor. Another anterior to the uterus. Patient became

[1] In all 157 cases. Of this number but 16 were found to be primary carcinoma of the ureter.

icteric without any accountable reason and died soon after. Clinical diagnosis, tumor of the ureter or rectum.

*Autopsy findings.* Upper portion of the ureter firm and hard; no mucous membrane left. At various places grayish yellow masses extend upward as far as the kidney. Grayish white strands pierce the muscular coats becoming continuous with the serosa. Many small nodules on the ascending colon. Mesenteric root filled with confluent cancer nodules. Peritoneum spotted with growths. Many subcapsular and interstitial nodules in the liver.

*Microscopic examination.* Medullary carcinoma of the ureter.

Case II. Davy (5). *History and symptoms.* Man, age 53 years. Injured in left testicle ten years previously. For two years pain in left loin and side. About five months ago passed a stone, size of a pea, followed by hematuria which has been intermittent since.

*Examination.* No urinary flow from the left ureter. Operation revealed a cyst on the left kidney, from which 33 ounces of fluid were removed. Wound healed and patient discharged. Returned three weeks later with extreme local pain. Left kidney was then removed. Probe meets obstruction four inches down from upper end of ureter. Wound healed and patient discharged. Death occurred five days later at an infirmary.

*Autopsy findings.* A growth completely obstructed the left ureter for a distance of five inches upward from the opening into the bladder. At the ureteral opening was a calculus the size of a hazel nut. The liver was studded with white cancerous masses, pea to walnut in size. Lumbar glands enlarged. Base of bladder invaded. Wall of rectum invaded with ulceration into the lumen. Probable cause, ureteral calculus.

*Microscopic findings.* Medullary carcinoma of the ureter.

Case III. Voelcker (16). *History and symptoms.* Man, age 68 years. Never sick except an attack of influenza two years ago. Patient noticed discoloration of his urine four months previous to entrance.

*Examination.* Edema of left ankle. Pain across loins. Nausea. Urine contained clotted blood. Liver enlarged, nodular and tender reaching to within two inches of the umbilicus. Resistance to palpation in the left iliac fossa. Patient rapidly losing weight. Liver rapidly enlarging. Death occurred six weeks after entry. No hematuria in the last few days.

*Autopsy findings.* Some jaundice. Both legs edematous. Colon

adherent to liver. Weight of latter 3118 grams. Left lobe infiltrated by pale soft new growth. Numerous nodules in right lobe. New growth in lower left ureter projecting into lumen as delicate villous processes covered with blood. Outside of ureter, mass the size of a cherry adherent to the pelvic brim. Left kidney showed hydronephrosis. Metastatic growths in the lymph glands to the left of the aorta. Nodule in right lung.

*Microscopic examination.* Ureteral growth was a villous carcinoma. Muscular layer invaded. Secondary growths similar in structure to those in the ureter.

Case IV. Hektoen (8). *History and symptoms.* Woman, age 50 years. Always in fair health. Eight months before death pains developed in the right hip. Lower extremity became swollen. Also swelling present in the right inguinal region. No history of injury.

*Examination.* Soft mass in right lower quadrant connected with the ileum. Diagnosis, osteosarcoma of the pelvis. Death due to exhaustion.

*Autopsy findings.* Tumor in the pelvis involving the right ureter. Right sided hydronephrosis. Probe passed into ureter from the bladder meets obstruction 2.5 cm. from the entrance. Upper portion of ureter entirely lost in tumor tissue. No metastases except paraureteral growth in the pelvis.

*Microscopic findings.* Typical medullary carcinoma. Many small islets of epithelial cells in the connective tissue.

Case V. Rundle (14). *History and symptoms.* Man, age 46 years. Year before admission patient noticed a fullness in the right abdomen which increased in size gradually and painlessly.

*Examination.* Fluctuating mass in lower part of abdomen, extends from a point two inches below the thorax to the middle line as well as far back into the lumbar region. Urine clear. No hematuria recently. No history of calculus. On several occasions the tumor was tapped. Fluid contained many granules and a few epithelial cells.

No clinical diagnosis given. Patient died from exhaustion.

*Autopsy findings.* Right sided hydronephrosis. Right ureter markedly dilated at its middle third. Lower third involved in a growth. Ureter nodular to within one inch of the kidney. Largest nodule four inches by two inches. Growth soft, white, and very friable. The left seminal vesical and vas more or less involved. Secondary deposits in the liver, lung and abdominal lymph glands.

*Microscopic findings.* Squamous celled epithelioma of the right ureter with extensive infiltration of its walls.

Case VI. Minich (11). Woman, age 66 years. (Report of a demonstration at autopsy; details lacking). Carcinoma of the right ureter. Lower third of ureter not patulous and adherent to the bladder and vagina. Perforation into vaginal canal admitting little finger. Perforation must be of recent date, for no changes have taken place in the vaginal wall as a result of urine trickling through the aperture.

Case VII. Gerstein (7). *History and symptoms.* Man, age 67 years. Periodic hemorrhage for nine months. Six months previous to entry diagnosis of renal hemorrhage was made. No normal urine for two months. Generally a dark red.

*Examination.* Skin somewhat icteric. Frequent micturition. Pain in the region of the right kidney. Cystoscope revealed a tumor in the region of the right ureteral orifice. It was furrowed; one of the clefts contained a blood coagulum and marked the exit of the ureter. A clinical diagnosis of malignancy was made. Operation showed an ulcerated tumor behind the right ureteral orifice. Removal out of the question. Patient died eight days later from heart failure.

*Autopsy findings.* Hydronephrosis of the right kidney. A tumor, pigeon egg in size, at the site of the right uretero-vesical junction. Ureter cannot be isolated from the tumor, tissues are too soft. Lower pole of corresponding kidney shows a metastatic nodule. Two nodules in right bladder wall. A pea sized nodule in right middle lobe of lung.

*Microscopic findings.* Section through tumor in ureter shows a typical carcinoma. Cells arranged in cords and nests. Ureteral lymph-vessels distended by carcinomatous cell masses. Secondaries show similar structure.

Case VIII. Adler (1). *History and symptoms.* Man, aged 69 years. For eight weeks severe backache, pains radiating toward the bladder. Urine periodically discolored.

*Examination.* Patient upon standing exhibits a stiff back. Vertebral column from tenth dorsal to the sacral vertebrae shows kyphosis. Urine brown. A clinical diagnosis was given as arteriosclerosis; degenerative myocarditis; chronic vertebral stiffness; tuberculous tumor (?) of the adrenal? (Addison's disease?). Patient died five months after entry from exhaustion.

*Autopsy findings.* Tumor of the left ureter 4 cm. from the vesical orifice, extending for 4.5 cm. along the ureter. Lower border well defined, upper border less so. Cut surface is grayish red, except cen-

*Tabulated data on all recorded cases of primary ureteral carcinoma*

| CASE NUMBER | REPORTED BY | DATE | SEX | AGE | CLINICAL SYMPTOMS | CLINICAL DIAGNOSIS | OPERATION | CAUSE | TYPE OF TUMOR |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Wising-Blix | 1878 | F. | 41 | Pain, lump in abdomen | Tumor of rectum | − | | Medullary carcinoma |
| 2 | Davy | 1884 | M. | 53 | Pain. Passed stone. Hematuria | Tumor found in relation to urinary tract after exploratory operation | + | Calculus | Medullary carcinoma |
| 3 | Voelcker | 1895 | M. | 68 | Hematuria 4 months | New growth | − | Calculus suggested; no evidence | Villous carcinoma |
| 4 | Hektoen | 1896 | F. | 50 | Pain. Swelling in hip | Osteosarcoma of pelvis | − | − | Medullary carcinoma |
| 5 | Rundle | 1896 | M. | 46 | Fullness in right abdomen | None given | − | − | Squamous celled epithelioma |
| 6 | Minich | 1902 | F. | 66 | None given | Details lacking | | | |
| 7 | Gerstein | 1902 | M. | 67 | Frequent hematuria | Malignancy of some sort | + | − | Typical carcinoma |
| 8 | Adler | 1905 | M. | 69 | Pain in back. Hematuria | Chronic vertebral stiffness. Tuberculous tumor? of adrenal? | − | Calculus may have lodged at lin. term | Papillary carcinoma |
| 9 | Metcalf-Safford | 1905 | M. | 47 | Frequent colicky attacks | Ureteral calculus | + | Calculus | Medullary adenocarc |
| 10 | Vorpal | 1906 | F. | 60 | Fell year ago; since then pain | Nephrosis from obstruction due to calculus or neoplasm | + | − | Medullary carcinoma |
| 11 | Richter | 1909 | F. | 80 | Pain. Hematuria Frequent micturition | Probably neoplasm of urinary tract | − | − | Papillary carcinoma |
| 12 | Zironi | 1909 | F. | 36 | Pain. Some hematuria Frequent micturition | Hydronephrosis from neoplasm, probably carcinoma | + | Calculus | Medullary carcinoma |
| 13 | Paschkis | 1910 | M. | 65 | Pain. Hematuria. Fluctuating tumor in abdomen | Hydronephrosis from malignancy of renal pelvis | − | Calculus | Papillary carcinoma |
| 14 | Israel | 1910 | F. | 60 | Severe pains. Hematuria. Icterus | Tumor of kidney or spleen or both | + | − | Papillary carcinoma |
| 15 | Chevassu-Mock | 1912 | M. | 53 | No pain. Hematuria for 5 weeks | Ureteral neoplasm | + | − | Epithelioma |
| 16 | Chiari | 1914 | F. | 54 | Loss of weight. Hematuria | Ureteral obstruction from lodged tissue or neoplasm | + | − | Papillary carcinoma |
| 17 | Butler | 1914 | M. | 53 | Pain. Loss of weight Hematuria | Sarcoma of ileum with involvement of ureter | + | − | Squamous celled carcinoma |
| 18 | Finsterer | 1915 | M. | 53 | Frequent hematuria | Vesical papilloma | + | − | Papillary carcinoma |
| 19 | Spiess | 1915 | F. | 41 | Pain in back, hips and pelvis | Dementia praecox | − | − | Medullary carcinoma |
| 20 | Schmitt | 1916 | F. | 55 | Pain in iliac region. Anuria | Intestinal obstruction<br>Tuberculous peritonitis suspected | + a yr. ago<br>− at present | − | Squamous celled carcinoma |

tral necrotic portion which is pale yellow. The fourth lumbar vertebra is infiltrated. Body of the bone is soft, and compressible between the fingers. No sign of bone can be seen; quite gelatinous. The site of the tumor is at the linea terminalis, where it is forced to make an angle in its descent. Probably resulted from the lodgment of a concretion at an earlier period.

*Microscopic findings.* Papillary carcinoma plus areas resembling squamous carcinoma. Vertebra sectioned without decalcification. Cells correspond to those in the original lesion.

Case IX. Metcalf and Safford (10). *History and symptoms.* Man, aged 47 years. Patient suffered from childhood with severe colicky attacks. Pain mostly in the left side of the abdomen. Attacks appeared every 2 or 3 years, sometimes remaining for weeks. Marked scoliosis due to sympathetic muscular contraction.

*Examination.* Urine showed pus cells, many reds and a few squamous cells. Skiagraphic examination negative. Cystoscopic examination attempted but there was too much hemorrhage due to an enlarged prostate. A clinical diagnosis of ureteral calculus was made from the frequent attacks of renal colic. An operation was advised. Nephrectomy and ureterectomy performed. Kidney showed pyelonephrosis, while a calculus was found in the lower end of the ureter. Three weeks later patient again developed intense pain, hence second operation for removal of growth extending from the point of ureteral separation to promontory of the sacrum. Death occurred from exhaustion nearly three months after entry.

No autopsy obtained.

Microscopic examination of the tissue removed showed primary adenocarcinoma of the ureter, medullary in character. Tissue removed from behind the bladder showed the same irregular mass of encephaloid cells. There can be no doubt of the etiological relation of the calculus.

Case X. Vorpal (17). *History and symptoms.* Woman, age 60 years. Patient fell on the right side over a year ago. Since then constant pain in back and down the right side. Five months later right sided nephroptosis was diagnosed. A tumor appeared at the same time increasing in size.

*Examination.* Tumor in right abdomen, size of a child's head. Did not move with respiration. The urine showed granular casts, epithelial red and white cells. Cystoscopic examination shows aperture to right ureter occluded by a growth the size of a cherry. There

was much and intense pain. A diagnosis of right sided nephrosis was made, resulting from obstruction of the ureter, due to a calculus, cicatricial stenosis or neoplasm.

Nephrectomy was performed. Ureter was dilated by a neoplasm at its lower end. Patient too weak to complete the operation. Death occurred 8 hours later with signs of embolus.

*Autopsy findings.* Aperture of the right ureter occluded by a projection the size of a hazel nut. Probe could be passed through its center into the ureter. Growth extended upwards for 5 cm.; 7 cm. farther up was a hard nodule similar to a scirrhus. Metastases were found in the retroperitoneal lymph glands and the liver. A nodule present in both the right kidney and right lung.

*Microscopic examination.* The tumor near the ureteral orifice appears as a medullary carcinoma, farther up the ureter it takes on the appearance of a carcinoma with cancroid pearls. Metastases similar in structure. In the liver especially both scirrhous and medullary types present.

Case XI. Richter (13). *History and symptoms.* Woman, age 80 years. Patient well until three months before entry. Then pain in back and right gluteal region. Frequent micturition. Urine blood stained.

*Examination.* Urine cloudy, reds and whites present. Also epithelial cells possessing the character of tumor cells, all in atypical division. No tumor visible with the cystoscope. The clinical diagnosis was a possible neoplasm of the urinary tract. Death occurred 14 days later.

*Autopsy findings.* A papillary swelling, hazel nut in size, was found in the right ureter 3 cm. from its vesical end, completely occluding the lumen. Metastases in the lymph gland at upper end of corresponding ureter, greyish red in color and having the consistency of bone marrow.

*Microscopic examination.* Many branching excrescences in the original lesion. In the deeper portions of the tumor there is an alveolar arrangement of the cells with atypical division. Tumor plugs in the dilated lymph vessels. Secondary in the lymph gland mostly alveolar in structure.

Case XII. Zironi (19). History and symptoms. Woman, age 36 years. Patient had more or less intermittent pains in the right lumbar region for 4 months.

*Examination.* Tumor in the right abdomen size of a child's head. By rectal and vaginal examination a cord the size of a finger can be

made out in the region of the right ureter pointing to the bladder. The urine contains neoplastic forms and a "pearl of Lebert." A diagnosis of hydronephrosis was made due to a neoplasm (probably carcinoma) in the lower part of the ureter.

Nephrotomy was performed. Upper end of the ureter was greatly dilated. Digital examination of this end shows a neoplasm the size of a walnut. Patient died nine days after the operation.

*Autopsy findings.* Right ureter throughout is a mass of neoplasms of varying sizes. Masses have enveloped the lumbar and hypogastric ganglia. Attached to the mucosa at the lower end of the ureter is a calculus whose center is softened containing epithelial elements. Metastases found in the paraureteral lymph glands. Neoplasm probably arose as a result of the chronic inflammation and irritation of the calculus.

*Microscopic examination.* Primary carcinoma of the ureteral mucous membrane.

Case XIII. Paschkis (12). *History and symptoms.* Man, age 65 years. Pains in the left lumbar region for some years. Severe pains with hematuria for 4 months.

*Examination.* Fluctuating tumor in the left abdomen. Urine bloody. Contains epithelium, cell detritus and leucocytes. Condition of patient did not permit an extensive examination nor operation. Clinically diagnosed as hydronephrosis resulting from malignancy of the renal pelvis. Death occurred 4 days later.

*Autopsy.* Enormous hematonephrosis of the left kidney. Ureter size of the small intestine at its upper end. At the level of the lower pole of the kidney to which it is attached, the ureter contains a tumor the size of a fist; 2.5 cm. lower down a calcium oxalate calculus the size of a bean, is lodged. Remainder of the ureter is normal. Metastases occur in the lymph glands, in the hilum of the kidney and in the retroperitoneal tissues.

*Microscopic findings.* Papillary carcinoma resulting from the irritation of the concretion.

Case XIV. Israel-Loewenstein (9). *History and symptoms.* Woman, age 60 years. Every 3 to 4 months within the last ten years the patient had excruciating pains arising in the left hypochondrium extending up into the chest and down into the thigh. Swelling, since the beginning of pain, which grew down the left abdomen. Frequent hematuria. Icterus with attacks of pain.

*Examination.* Skin slightly icteric. Palpable tumor in the left

abdomen size of a child's head. An immovable parenteral mass felt on examination per vagina and per rectum. A diagnosis of a tumor of the kidney or spleen was made. Operation was advised. A hydronephrotic kidney and an enlarged spleen were removed. Blood pressure dropped and the patient died the same day.

*Autopsy findings.* In lower and upper end of the ureter many papillomatous outgrowths into the lumen of the ureter. Between the two a definite tumor infiltrating the paraureteral tissue. Ureter more or less infiltrated throughout. A metastatic nodule present in the left parametrium involving the trunk of the sacral plexus. This one palpable per vagina. Nodule in the right kidney.

*Microscopic findings.* Many of the projections were papillomatous with carcinomatous changes showing cell cords and nests. Lymphatics involved.

Case XV. Chevassu and Mock (3). *History and symptoms.* Man, age 53 years. Hematuria more or less intermittent for 5 weeks; no pain.

*Examination.* Clinical examination negative. Cystoscopic examination shows punctiform ureteral openings. Bloody ejaculations from the left. Catheter in the left ureter meets two obstructions in the lumen with bleeding. Clinical diagnosis—ureteral neoplasm. Operation performed for removal of both the kidney and ureter on the left side. Patient recovered.

There were two nodules in the removed ureter, one having perforated the ureteral wall. Has an embossed appearance and the cut surface is yellow and lardaceous in appearance. A white granulation under capsule of the kidney. No metastases evident.

*Microscopic findings.* Primary epithelioma of the ureter. Plain muscle was infiltrated. Small nodules were situated in relation with large vessels. Others gave appearance of having developed in the lymph vessels. Paraureteral tissues free. Nodule in kidney was an adenoma.

Case XVI. Chiari (4). *History and symptoms.* Woman, age 54 years. Hematuria three months before entrance; again two weeks before entrance. Decrease in weight.

*Examination.* No tumor mass; no tenderness. Urine contains a few reds and a few whites. Cystoscope shows absence of urinary flow from the left ureter; only a few drops of blood exude. Probe in left ureter meets obstruction 8 cm. from the bladder. The clinical diagnosis of obstruction of the left ureter was made, due to lodgment

of tissue from the kidney or a neoplasm at the site of obstruction. Operation advised. Nephrectomy and ureterectomy performed. A tumor the size of a "nut" was found in the ureter 10–12 cm. from its lower end. The ureteral wall was bound to the paraureteral tissues.

Patient recovered and was well one year after the operation.

The ureter shows a papillary tumor the size of a cherry whose base nearly encircles the wall of the ureter. From its warty surface a projection 2 cm. in length hangs into the lumen of the ureter. Wall more or less infiltrated.

*Microscopic examination.* Papillary carcinoma. The carcinomatous cell nests have infiltrated the musculature of the ureteral wall. Growth has not spread beyond the ureter.

Case XVII. Butler (2). *History and symptoms.* Man, age 53 years. Hematuria nineteen and again seven months previous to entrance. Later incontinence. Much pain. Loss in weight.

*Examination.* Complains of sciatica. By palpation a firm mass 3 inches in diameter is felt to the right of the umbilicus. Tenderness in the right sacroiliac region. Catheter in the right ureter meets obstruction 6 cm. from the orifice. No urine from right catheter. Right testicle enlarged. The clinical diagnosis was sarcoma of the right ileum with secondary involvement of the ureter. An operation advised. A tumor was found behind and below the kidney spreading over the surface of the ileum and sacrum. Had the appearance of a sarcoma. A small portion was excised for microscopic examination. The testis was considered a metastatic growth and removed but contained only normal tissue. Patient gradually declined and died two weeks later.

*Autopsy findings.* Large tumor mass obliterating the central half of the right ureter. Another lesion at the lower end of ureter. There was infiltration of psoas and iliac muscles, perirenal tissues, retroperitoneal tissues and the lumbar plexus.

*Microscopic findings.* The diagnosis was changed to primary carcinoma of the ureter after examination of the excised tissue. The mass was made up of solid areas of squamous cells. Small amount of connective tissue. Some epithelial whorls.

Case XVIII. Finsterer (6). *History and symptoms.* Man, age 53 years. Bloody urine 4 years ago. Oft repeated.

*Examination.* Hemorrhage from urethra. Diagnosis was that of vesical papilloma.

Operation showed a papilloma of the left trigone the size of a pigeon

egg and extending up the left ureter. Ten cm. of the ureter was removed and the stump sutured to the bladder. Recovery.

*Microscopic findings.* Beginning carcinomatous degeneration of ureteral papilloma.

Case XIX. Spiess (15). *History and symptoms.* Woman, age 41 years. Pleurisy twice. Angeitis in left leg. For three weeks pain in back, hips, pelvis, radiating into the right thigh.

*Examination.* Abdomen not sensitive. No palpable tumor mass. Rectal and vaginal examination negative. Patient very nervous, but improved and discharged. Diagnosis: anemia and hysteria. Patient reentered in two weeks. Nervous symptoms persistent. Death in two days. Diagnosis, Dementia praecox.

*Autopsy findings.* Tumor in the right ureter 3 cm. from the bladder and 1.5 cm. in thickness; remainder of ureter more or less infiltrated. Paraureteral tissue raised in nodules protruding forward into the peritoneal cavity but covered by peritoneum. Many yellowish white masses extending from iliac fossa to the kidney. Both psoas muscles invaded. Aorta and inferior vena cava surrounded by growths. Subpleural lymph-vessels of upper and right lower lobes filled with tumor masses. Lung substance of both upper lobes involved. Glands at hilus enlarged. Metastases also in the right fallopian tube, mesenteric, retroperitoneal and left axillary nodes, and in the cysterna chyli.

*Microscopic findings.* Ureteral carcinoma. All metastases similar.

To the above cases already recorded is added the following new case.

XX. *History and symptoms.* Woman, age 55 years. The patient was admitted to Cook County Hospital on August 24, 1915, brought in an ambulance.

The following history was elicited: Complains of diffuse pains in the abdomen. Is constipated, has had no movement for three days; no nausea, vomiting or headache. Five weeks ago the patient was seized with severe pains in the left abdomen low down in the iliac region; three days later she began vomiting and has vomited considerably ever since; was in bed constantly for the last four weeks; for the past two weeks has had diffuse pains in the abdomen; abdomen tender; constipated for nine months; has used cathartics for three months; headache after vomiting spells; pain and soreness in the back in region of

the left iliac bone for five weeks. Operated on at another hospital last December for tumor, abscess and "decayed ovary." Married 18 years; husband is alive. No venereal disease. Rheumatism since she was forty-five. One miscarriage; curetted at 35 for dysmenorrhea; has not menstruated for 6 years.

Examination revealed marked anemia; many teeth missing, tongue coated, larynx negative. Urine negative as regards albumin and casts; later examination shows hyaline and granular casts. Blood count shows 23,800 white corpuscles. Operation showed many adhesions about the intestines.

Clinical diagnosis of intestinal obstruction was made. Tuberculous peritonitis was suspected. Patient died November 5, 1915, from exhaustion. Autopsy was performed by Dr. H. Gideon Wells.

*Autopsy.* External appearance. The body is that of a tall, poorly nourished woman; the skin is white, no icterus, pallor about the nipples. Healed abdominal incision in the midline from the umbilicus to the pubis. Superficial lymph glands are not palpable. No subcutaneous edema. There is a superficial excoriation of the skin about the buttock on each side of the anus. Subcutaneous fat dark in color.

Abdominal cavity. Omentum diffusely adherent to anterior abdominal wall, many diffuse adhesions on the right side binding the intestines together; many blackish spots on the parietal peritoneum; several loops of the intestine are bound together in a solid mass; dense adhesions bind the stomach to the liver; the latter is also adherent to the diaphragm; firm fibrous adhesions about the gall bladder, inguinal and femoral rings closed; no acute inflammation; adhesions are dense in the pelvis and on the right costal margin; to the left of the median line there is a large retroperitoneal mass to which the upper part of the jejunum is firmly adherent and sharply kinked; the colon is not involved; the mass is apparently related to the kidney. The spleen is embedded in adhesions; it is not related to the tumor mass. There is a fresh blood clot in the pelvis in the region of the sigmoid, also many adhesions here; the bladder is moderately distended; the uterus firmly adherent to the parietal peritoneum

at the center of the left pelvis; left ovary and tube have been removed; no evidence of obstruction of the bowels; no gangrene.

Pleural cavity. There was no excess fluid; no adhesions except at right lower lobe which is adherent to the diaphragm.

Pericardial cavity is normal.

Heart. Weight, 240 grams. Coronary arteries not unduly prominent and normal in size. Beginning of aorta shows slight sclerosis and slight dilatation; thoracic aorta shows no abnormalities. All valves normal. Cardiac walls slightly atrophied.

Lungs. Small and inelastic. Right, weight 280 grams. At hilum there is a small calcified bronchial gland; organ very light; no areas of consolidation; near the base is a small white calcified nodule, 2 mm. in diameter. Left, weight 200 grams, presents the same general appearance.

Liver. Weight, 1140 grams. Small, a few calcified nodules in the adhesions; a white nodule in the left lobe, 7 mm. in diameter, which extends 3 mm. into the liver tissue; also one in the right lobe 2 mm. in diameter; the cut surface shows another of the same appearance. The gall-bladder is distended and under tension; contains 16 small concretions, dark in color, average 2–3 mm. in diameter; they are quite firm, have a light colored center; common and hepatic ducts normal.

Spleen. Weight, 100 grams. Approximately normal in size and consistency.

Stomach and intestines are normal.

Pancreas. For a distance of about 3 cm. down from the head are small areas where the fibrous tissue is slightly increased; there are also a number of whitish necrotic spots extending into the pancreas.

Adrenals. Both are normal except for post mortem changes.

Kidneys. Right, a little firmer than normal, capsule somewhat adherent; when latter is removed leaves a slightly granular surface; the pelvis and ureter are not distended. Left, perirenal tissue more fibrotic than normal. Pus is found under pressure in the distended pelvis and the kidney is slightly atrophied, cortex and medulla together measuring 10–15 mm. The kidney itself is not involved by necrotic tissue except in the

floor of the pelvis; one white nodule 5 mm. in diameter in the cortex; consistency diffusely increased and capsule firmly adherent.

Bladder. The mucous membrane is granular around the urethral opening; otherwise normal.

Generative organs. The right ovary, the only one remaining, is normal. The uterus is adherent to the pelvic wall on the left side, rather small, and there is an increase of whitish tissue in the submucosa; an encapsulated firm nodule, 1 cm. in diameter, is embedded in the anterior wall just above the cervix; the cervix is distended to 8 mm. diameter by a plug of viscid mucus at the external os. Vagina normal.

Tumor mass. A tumor mass in the left abdominal cavity and in relation to the left kidney is very firmly adherent to the abdominal wall and to the anterior wall of the vertebral column from the eleventh dorsal to the third lumbar vertebra. This mass is fluctuating and when ruptured there issues a purulent cloudy fluid, containing shreds of necrotic tissue. The dorsal boundary is quite necrotic, the growth having invaded the bodies of the vertebrae, that of the third being almost destroyed. The growth also infiltrates but does not follow the psoas muscles. When opened it is found to consist of a cavity about 10 cm. in diameter and 15 cm. vertically, with walls of a friable pinkish shredded tissue on a firm white base, totalling 2–3 cm. thick in most places. Laterally this wall is well defined but medially it involves the vertebral column by infiltration to such an extent that it cannot be separated from the bone. The upper boundary is somewhat above the junction of the ureter with the pelvis of the kidney. The origin of the ureter (fig. 1 $ur_3$) is occluded by cancer tissue, causing a dilatation of the renal pelvis. The lower boundary is continuous with ureter, which is found extending as an occluded, attenuated and infiltrated solid cord about 3 cm. within the cavity ($ur_2$). The ureter below this point, where it makes its exit from the cavity, is normal ($ur_2$). Regional lymph glands do not seem to be involved, but there is some diffuse neoplastic tissue about the aorta and cava, binding these vessels and the prevertebral tissue into a solid mass with the ureteral growth.

Histological findings. The microscopic findings confirm the anatomical diagnosis—transitional celled carcinoma with primary origin in the ureter; the latter was obliterated and the retroperitoneal tissues quite extensively invaded. Metastases


FIG. 1. Posterior view of cancer showing the necrotic cavity. *aa*, abdominal aorta; *kid*, kidney; $ur_1$, ureter at its exit from the necrotic cavity; $ur_2$, upper attenuated end of lower segment of ureter lying free in the necrotic cavity; $ur_3$, portion of occluded ureter in union with the kidney.

were found in the portal canal of the liver, under the capsule of the left kidney, and in the bodies of several vertebrae; the latter showed an invasion of the bone trabeculae by the tumor

cells, also a decrease in the hemopoetic function of the narrow and quite extensive necrosis; no hornification or tubule formation of the epithelium.

The lungs showed senile emphysema; the liver, fatty changes; in the spleen slight pigmentation and fibrosis. Fatty infiltration was noted in the pancreas and early fat necrosis with beginning deposition of calcium. The right kidney showed hyaline casts, some interstitial and hemorrhagic nephritis and a few hyaline glomeruli. The left kidney, besides the neoplasm, showed parenchymatous atrophy, fibrosis and many leucocytes. The ovary, besides showing the corpora albicantes, presented typical senile changes and the absence of Graafian follicles. The submucosa of the uterus was quite hyperplastic and in its wall was found an interstitial uterine fibromyoma which possessed a good deal of hyaline connective tissue.

Of the 20 cases here tabulated the sexes are equally represented numerically. The average age is 55.8 years, that for the female being slightly lower, 54.3 years. The youngest patient was a woman of 36; the oldest, a woman of 80. Most of them are accidental findings at autopsy, a few are diagnosed clinically.

The symptomatology gives rise to a few interesting points: The onset of the trouble was invariably insidious and painless, the first evidence being usually a swelling in the abdomen noticed by the patient, which is generally attributable to a hydronephrosis due to ureteral obstruction. Hematuria takes place almost without an exception and may be intermittent or continuous and extend over a period of years. Pain is often severe and intense, but appears late in the course of the disease.

No very satisfactory conclusions as regards the etiology of these tumors can be drawn from the table. In four cases calculi were found; in two other cases calculi were suspected to have been present at some time antedating the trouble. When present, they occur, as pointed out by Spiess, at one of three anatomical regions of the ureter, viz., at its origin, at the angle where it crosses the linea innominata and at the narrowed portion before entering the bladder.

Operations were performed on 10 patients with 3 recoveries.

These were done either for exploratory purposes, thus helping to establish a diagnosis by removal and examination of the pathological tissues, or for removal to relieve pain. Death in a few cases was immediately postoperative, in the remainder it was due to exhaustion.

All these cases, except the three recoveries where the lesion did not extend beyond the ureteral musculature, showed quite extensive infiltration and often necrosis of the surrounding tissues. Metastases occurred in nearly all the cases, the lung and liver frequently being the seat of these secondary growths. One case showed tumor thrombi in the neighboring blood-vessels, while most of them showed metastases in regional lymph glands, and tumor nodules and thrombi in the lymph vessels draining the part. Of the cases reported as recoveries it was too early to state at the time reported whether there was a recurrence, although one patient, that of Chiari, was well one year after the operation.

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# THE INHERITABILITY OF SPONTANEOUS TUMORS OF SPECIFIC ORGANS AND OF SPECIFIC TYPES IN MICE[1]

## STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

### Fifth Report

MAUD SLYE

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During the past three years I have published from this laboratory a series of papers giving data and charts which demonstrate the persistent tendency toward the occurrence of spontaneous tumors in strains of mice where tumorous individuals have been bred in.

(1) This persistence occurs when given the only two possible tests of heredity, namely, inbreeding and hybridization; and it occurs in *every* such strain where the offspring have lived to cancer age in sufficient numbers to make even a meager test.

(2) On the other hand, certain strains of mice when given every possible test by the closest inbreeding and by hybridization with other proved non-cancerous strains have never produced a single case of tumor of any sort.

And (3) into such proved non-tumorous strains of mice, cancer has been introduced by hybridizing them with proved tumor-bearing strains. From such crosses, in every case where the offspring have lived to cancer age in sufficient numbers to make a test, it has been possible to extract, on the one hand, strains which

[1] Presented before the American Association for Cancer Research, St. Louis, Mo., April, 1915, and before the Second Pan-American Scientific Congress, Washington, D. C., January, 1916.

never produce cancer; and, on the other hand, strains which inevitably do produce cancer and which in turn carry it into every strain with which they are hybridized.

Moreover, the behavior of spontaneous cancer in heredity is shown in these reports to be strikingly like that of a mendelian so-called "recessive," such as albinism. A summation of the results in Chart 21 of the third report shows a very close approximation to the "mendelian expectation" for a recessive (3). At the same time control experiments were described, by which other possible causative factors for the transmission of spontaneous cancer have been eliminated (1, 3).

It might be well to repeat at this time that the tumors reported are all spontaneous tumors, arising in the ordinary course of mouse life, without any artificial interference whatever in the nature of grafts, inoculations, or any other form of manipulation. The stock is kept under conditions of the most rigorous hygiene; and every effort is made to eliminate infections and to carry every individual to the greatest possible age.

The housing, feeding, and every other detail in the life of the mice is identical in the tumor and in the non-tumor strains, thus eliminating these external conditions as a factor in the occurrence of cancer within certain strains and its absolute non-occurrence in certain other strains. It is an interesting point that during the ten years of the maintenance of this stock there has never been a severe epidemic of any type whatever, not even of intestinal or pulmonary infections, to which mice are easily subject.

The routine which has been established in the laboratory in the matter of housing, food, reproduction, etc., has yielded a stock whose average age is far beyond the average age limit quoted for mice. Many individuals both in the tumor and in the non-tumor strains live to be from three to five years old. The strains are vigorously reproductive and bear inbreeding for many generations before they run out.

The ten years of hygiene behind this stock, which have resulted in long-lived and highly reproductive strains, undoubtedly have made it possible to secure many strong strains from can-

cerous ancestry, whose individual representatives live in large numbers well into cancer age, and which bear the test of inbreeding through many generations.

The present study is based on a living population of about twelve thousand, which has been held at that number for the past two years. During that period there has been a steady output from the cancer bearing strains of from fifty to one hundred cases of spontaneous cancer at any given time. The study is based upon over fourteen thousand autopsies, which include over fifteen hundred cases of tumor, involving over twenty-five hundred individual primary neoplasms.

Among these cases there have been over four hundred secondary tumors, not counting multiple nodules in a given organ in any given case. In other words, over 40 per cent of the cases have shown metastasis.

From this background of general cancer study, data have now arisen on the subject of the influence of heredity upon spontaneous tumors of specific organs and of specific types, when given the same tests of inbreeding and of hybridization.

It must be emphasized that a study of the inheritability of tumors of specific organs and of specific types can not be undertaken until one has accumulated a vast amount of data on a very extensive tumor-bearing stock; for with the exception of mammary gland tumors and of lung tumors (which together comprise about 90 per cent of all spontaneous mouse tumors) the cases arise slowly. There is at hand at the present time, however, enough material for the study of a considerable number of tumors of different types and of different organs. It is the purpose of this report to make a general survey of that field in order that the results may not be selective, but may demonstrate the general facts. Let me repeat at this point that the facts demonstrated apply only to the spontaneous tumors with which they deal.

The cancer-bearing strains in this laboratory (except a few wild strains of both *Mus* and *Peromyscus*) all arise from three original and wholly unrelated stocks:

(1) A stock of albinos secured in Illinois over ten years ago,

represented in this work by Strain 94. Only a little of this stock remained when the study of cancer was begun, but it furnished a few tumors, all of them of the mammary gland or of the lung.

(2) A stock of the Japanese white-footed mouse (Strain 90 in these studies), a grey-white piebald mouse secured direct from Japan. It had been carried through many generations, and its representatives, when these studies began, were scattered through various generations with but few individuals remaining in any given generation. It was prolific, however, and had been extensively hybridized with many different strains. The percentage of cancer was fairly high, and the tumors exhibited great diversity in their location. There have been in this strain malignant growths in the mammary gland, lung, liver, kidney, ovary, testicle and mediastinum.

It is a fact that strongly substantiates the theory of the inheritability of the cancer tendency, that, aside from the common mammary gland and lung tumors (one or both of which have come in with every stock of cancer mice which I have handled), all the tumors of the series, with the exception of four ovarian tumors, a few mediastinal lymphomas and tumors of the jaw, and two testicle tumors, have arisen in this strain and its derivatives, although other strains, both cancerous and non-cancerous, have been carried side by side with it and its derivatives for ten years, subjected to exactly the same influences, handled by exactly the same methods, living a life of exactly the same routine.

In early work when this strain and its immediate hybrids were numerous, there were many tumors other than those of the mammary gland and of the lung. Then there was a period during which very few such tumors arose. Now, however, when members of the late hybridizations of these old strains are reaching well into cancer age, these tumors of the different organs are appearing again.

For example, eight out of fourteen ovarian tumors occurred in the first twenty-two hundred autopsies; the remaining six, in the last three thousand. Through the intermediate four thousand autopsies there was not one case of ovarian tumor.

Again, in the liver tumors, through an intermediate period of

five thousand autopsies there were less than one half the number occurring in the second thousand alone. But since the late hybrids of these old liver tumor strains are coming into cancer age, over 50 per cent of the entire number of liver tumors has arisen, and it must be emphasized that they have arisen in the direct descendants of the old liver tumor mice whose death long antedated even the conception of these recent bearers of liver tumors.

(3) The third general cancer producing stock was made up of pure-bred albino and self-colored pigmented strains secured from the breeding establishment at Granby, Mass. This stock, in my hands for nearly ten years, has rarely produced anything but mammary gland and lung tumors either in inbreeding or in hybridization with other stock from the same source.

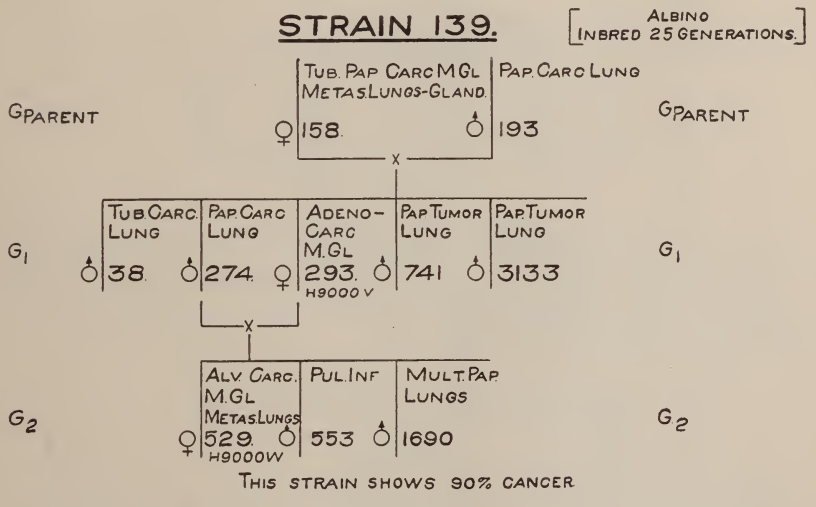



CHART 1

TUMORS OF THE LUNG (4)

*Chart 1*

Note Strain 139, in which every member that lived to lung tumor age[2] except one had a tumor of the lung, primary or sec-

[2] Spontaneous lung tumors rarely arise in this stock in individuals under one year of age.

ondary, nearly all of them malignant. Male No. 553 died at six months of age. The other tumors represented in this strain are three cases of mammary gland carcinoma.

*Chart 2*

*Strain 280.* In this strain both parents had primary carcinoma of the lung. The strain carries 100 per cent of tumors, 80 per cent of which are tumors of the lung, primary or secondary.

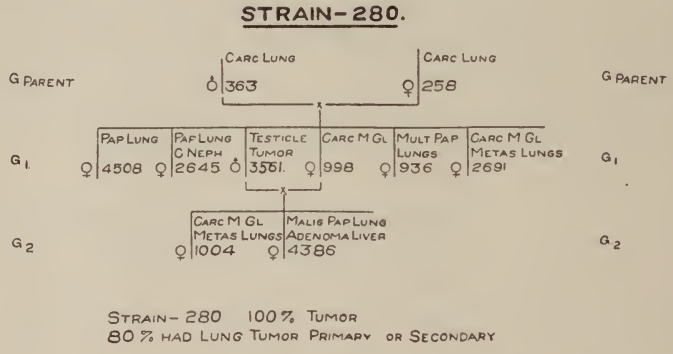



CHART 2

In these two typical instances, then, where lung tumor existed in both parents, lung tumor occurred in a strikingly high percentage of the offspring through as many generations as the strain was carried.

## TUMORS OF THE MAMMARY GLAND

*Chart 3*

*Strain 65.* The parent female of this strain was a member of Strain 139 which carried only lung and mammary gland tumors. The parent male came from a branch of Strain 90 which produced many mammary gland tumors and a few lung tumors. The resulting strain, represented by ninety-seven members in six generations, shows over 28 per cent of the members cancerous. Of these tumors 75 per cent arose in the mammary gland, and 14 per cent in the lung, with two cases of thymus tumor in one

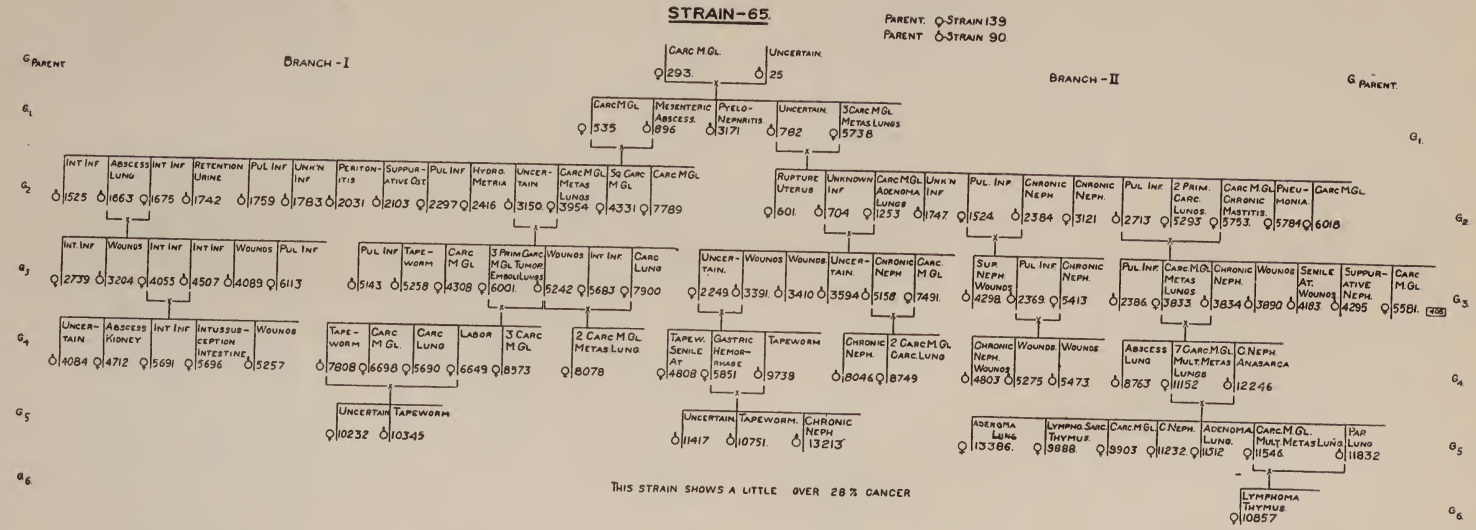

STRAIN-65.
PARENT ♀-STRAIN 139
PARENT ♂-STRAIN 90
G PARENT
BRANCH - I
BRANCH - II
G PARENT
G1
G2
G3
G4
G5
G6
CARC M GL. ♀293. UNCERTAIN. ♂25
CARC M GL ♀535 | MESENTERIC ABSCESS ♂896 | PYELO-NEPHRITIS ♂3171 | UNCERTAIN. ♂782 | 3 CARC M GL METAS LUNGS ♀5738
INT INF ♂1525 | ABSCESS LUNG ♂1663 | INT INF ♀1675 | RETENTION URINE ♂1742 | PUL INF ♀1759 | UNK'N INF ♂1783 | PERITONITIS ♂2031 | SUPPURATIVE OT. ♂2103 | PUL INF ♀2297 | HYDRO. METRIA ♀2416 | UNCERTAIN ♂3150 | CARC M GL METAS LUNGS ♀3954 | SQ CARC M GL ♀4331 | CARC M GL ♀7789
RUPTURE UTERUS ♀601. | UNKNOWN INF ♂704 | CARC M GL ADENOMA LUNGS ♀1253 | UNK'N INF ♂1747 | PUL. INF. ♀1524 | CHRONIC NEPH ♂2384 | CHRONIC NEPH. ♀3121 | PUL INF ♂2713 | 2 PRIM. CARC. LUNGS. ♀5293 | CARC M GL CHRONIC MASTITIS ♀5753. | PNEUMONIA. ♀5784 | CARC M GL. ♀6018
INT. INF ♀2739 | WOUNDS ♂3204 | INT INF ♀4055 | INT INF ♂4507 | WOUNDS ♀4089 | PUL INF ♂6113
PUL INF ♂5143 | TAPEWORM ♂5258 | CARC M GL ♀4308 | 3 PRIM CARC M GL TUMOR EMBOLI LUNGS ♀6001. | WOUNDS ♂5242 | INT INF. ♀5683 | CARC LUNG ♀7900
UNCERTAIN. ♀2249 | WOUNDS ♂3391. | WOUNDS ♀3410 | UNCERTAIN. ♂3594 | CHRONIC NEPH ♀5158 | CARC. M GL ♀7491.
SUP NEPH WOUNDS ♀4298 | PUL INF ♂2369. | CHRONIC NEPH. ♀5413
PUL INF. ♀2386. | CARC M GL METAS LUNGS ♀3833 | CHRONIC NEPH. ♂3834 | WOUNDS ♀3890 | SENILE AT. WOUNDS ♂4183 | SUPPURATIVE NEPH. ♀4295 | CARC M GL. ♀5581. 406
UNCERTAIN. ♀4084 | ABSCESS KIDNEY ♂4712 | INT INF ♀5691 | INTUSSUSCEPTION INTESTINE ♂5696 | WOUNDS ♀5257
TAPEWORM ♀7808 | CARC M GL. ♂6698 | CARC LUNG ♀5690 | LABOR ♂6649 | 3 CARC M GL ♀8573 | 2 CARC M GL METAS LUNG ♀8078
TAPEW. SENILE AT ♀4808 | GASTRIC HEMORRHAGE ♂5851 | TAPEWORM ♀9738
CHRONIC NEPH. ♀8046 | 2 CARC M GL CARC LUNG ♀8749
CHRONIC NEPH. WOUNDS ♀4803 | WOUNDS. ♂5273 | WOUNDS. ♀5473
ABSCESS LUNG ♀8763 | 7 CARC M GL MULT. METAS LUNGS ♀11152 | C. NEPH. ANASARCA ♀12246
UNCERTAIN ♀10232 | TAPEWORM ♂10345
UNCERTAIN. ♀11417 | TAPEWORM. ♂10751. | CHRONIC NEPH ♀13213
ADENOMA LUNG ♀13386. | LYMPHO. SARC THYMUS. ♀9888. | CARC M GL. ♀9903 | C. NEPH. ♀11232 | ADENOMA LUNG. ♂11512 | CARC. M GL. MULT. METAS LUNG. ♀11546. | PAP LUNG ♀11832
LYMPHOMA THYMUS. ♀10857
THIS STRAIN SHOWS A LITTLE OVER 28% CANCER

CHART 3

branch of the family (aunt and niece), and no other tumor of any other organ.

Strains 73, 264, and 190, published in the second report of these studies, produced little beside mammary gland tumors. Strain 146 (3), its progenitors having been derived from strains which carried mammary gland and lung tumors, bred through eight generations and in thirteen branches, has rarely produced anything but mammary gland and lung tumors.

Strains 304, 343, 413, etc. (3), hybrid derivatives of Strain 146, also produced few tumors except of the mammary gland and of the lung. That is, where the ancestry of a strain carries predominantly mammary gland tumor, the resulting strains (which are not selected, but absolutely typical) furnish little but mammary gland tumors when inbred; and in hybridization also the resulting strains show a heavy percentage of mammary gland tumors.

*Chart 4*

*Strain 450.* Chart 4 shows Strain 450 with its complete ancestry. *On the maternal side* there are five consecutive generations of tumorous parentage, in all except one of which both parents were tumorous; in this single instance (male No. 242) there was cancer in the ancestry. In this maternal ancestry there were five generations of mammary gland tumor, all but one (a sarcoma) carried by the female; there were four generations of primary lung carcinoma, two in females and two in males; there was one case of leukemia, and one case of sarcoma of the testicle, both in males.

The resulting female No. 5924, maternal parent of Strain 450, had a primary carcinoma of the mammary gland and a primary carcinoma of the lung.

*On the paternal side.* There were three generations of carcinoma behind the parent male, Male No. 5183, who did not himself have tumor, but who died young of an intestinal infection and a hypertrophied heart.

Strain 450 was produced by the combination of seven strains;

namely 138, 139, 146, 151, 90, 143 and 85, all of which carried mammary gland tumors. Strains 139, 146, 151 and 90 carried lung tumors. Strains 146 and 138 carried leukemia and pseudo-leukemia. Strain 90 carried tumors of the kidney.

The resulting Strain 450 gives nine individuals who lived to six months or more, among them being five cases of carcinoma of

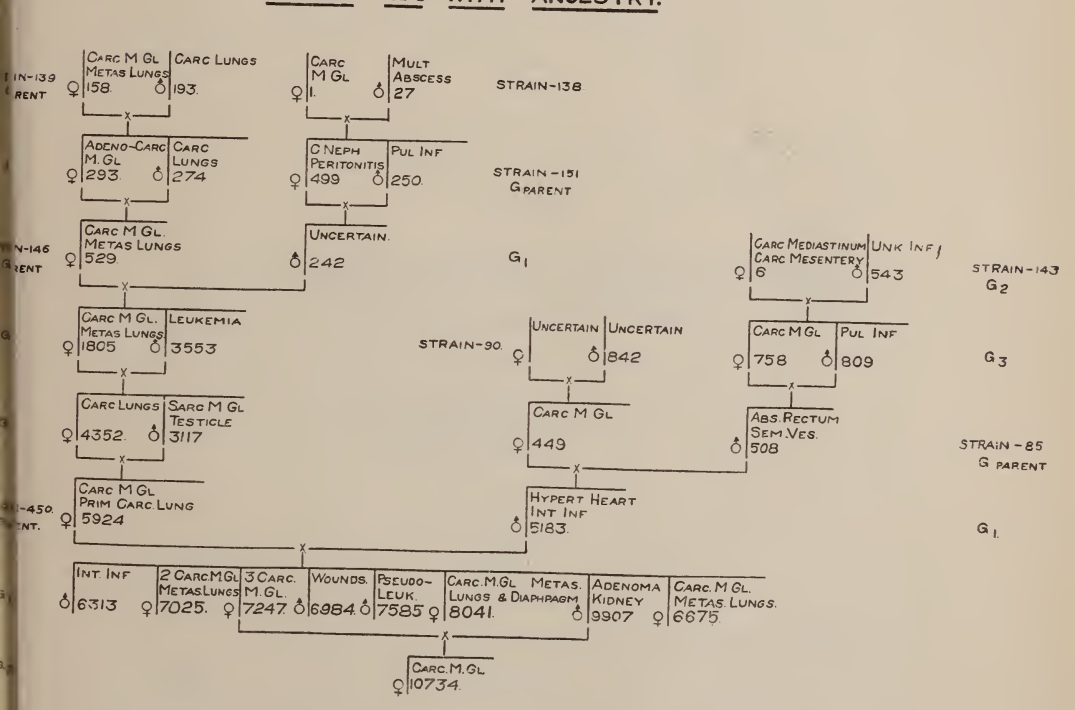



CHART 4

the mammary gland, all but two with secondaries in the lungs, one case of pseudoleukemia, and one of adenoma of the kidney. That is, every type of tumor which entered into the ancestry of this particular strain is represented in the resulting strain; and, on the other hand, no type whatever, other than those which entered into the ancestry, is represented in this strain.

TUMORS OF THE OVARY[3]

*Chart 5*

*Strain 196.* Chart 5 is so drawn as to cut out all fraternities in which ovarian tumor did not arise. This is done in order to get

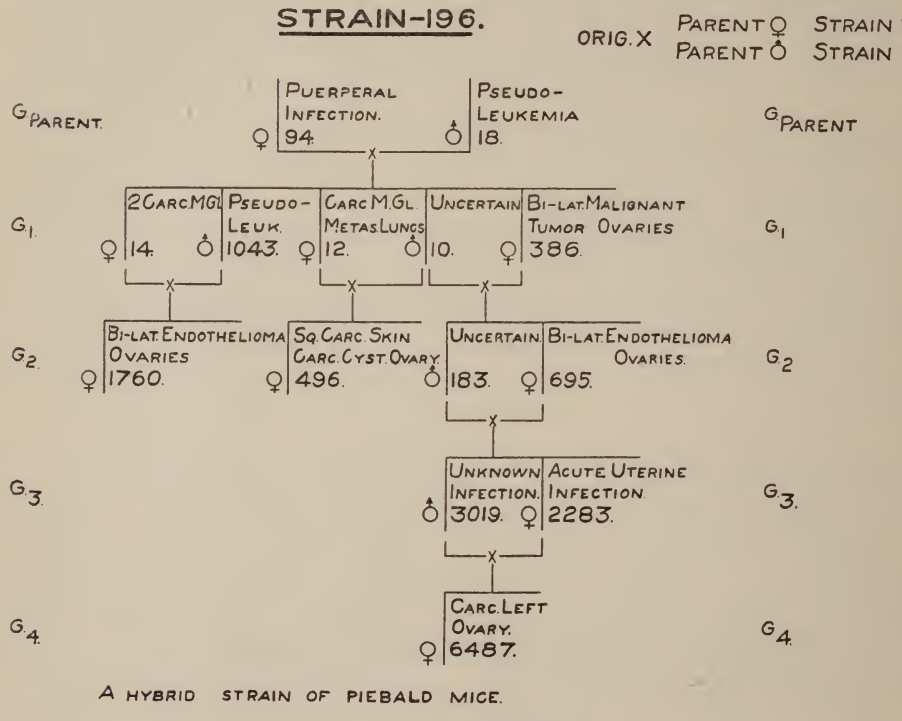



CHART 5

the strain within the limits of a single chart and to show where the ovarian tumors occurred. Five out of the entire eighteen ovarian tumors of the first eleven thousand autopsies fell in this

[3] The character of the ovarian tumors is merely indicated by the terms used (carcinoma, endothelioma). This material will be studied more extensively later (as has been done with the lung and liver tumors and the sarcomas), and the designations used may be changed in some instances. The growths included, however, are all unquestionable neoplasms, many of them of identical structure within a given strain. These are the points of significance in the present discussion.

strain. All the others except four occurred in hybrid strains derived from or allied to Strain 196. For example, female No. 9137, with a so-called endothelioma of the ovary, was the granddaughter of female 1760 in a hybrid cross (shown in Chart 7, Strain 201). That is, Strain 196, both in inbreeding and in hybridization, produced ovarian tumor. Of all the ovarian tumors arising in the first eleven thousand autopsies, over 77 per cent fell in Strain 196, its derivatives, and allied strains. This is a striking incidence within a strain and its derivatives, when one considers the rarity of ovarian tumors in mice.

## TUMORS OF THE LIVER (5)

### *Chart 6*

*Strain 202.* Chart 6 has been drawn to show both the maternal and the paternal ancestry that lay behind Strain 202, and includes the fraternities showing tumors of the liver either primary or secondary. Its object is to show where the liver tumors, both primary and secondary, occurred in this strain and its ancestry. The rest of the fraternities are necessarily excluded in order to get the data within the limits of a chart. With a high percentage of liver tumor on the maternal side, and with the paternal side of the ancestry derived from the same old stock as the maternal ancestry, namely, Strain 90 and Strain 94, the resulting Strain 202 shows over 23 per cent of liver tumor in mice which lived to cancer age, although neither immediate parent had liver tumor, both having died young, and although these liver tumor members of the strain were not conceived until after the death of their liver tumor ancestry. This is an extremely high percentage considering the great rarity of liver tumors. Outside of this stock there has been but one case of liver tumor reported in all mouse literature. This one case was reported from the Imperial Cancer Research Fund of London (6).

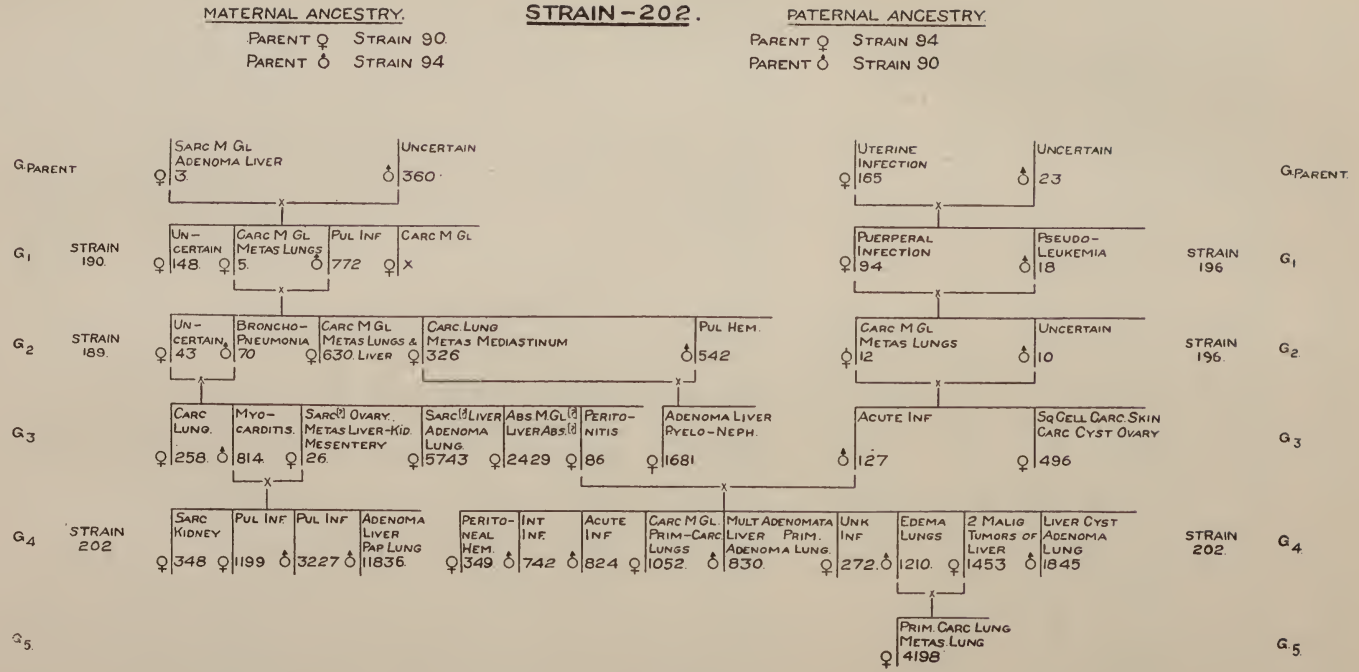

MATERNAL ANCESTRY.
PARENT ♀ STRAIN 90.
PARENT ♂ STRAIN 94
STRAIN-202.
PATERNAL ANCESTRY
PARENT ♀ STRAIN 94
PARENT ♂ STRAIN 90
G. PARENT
G1
G2
G3
G4
G5
STRAIN 190.
STRAIN 189.
STRAIN 202
STRAIN 196
STRAIN 196.
STRAIN 202.
Sarc M Gl Adenoma Liver ♀ 3.
Uncertain ♂ 360.
Un-certain ♀ 148.
Carc M Gl Metas Lungs ♀ 5.
Pul Inf ♂ 772.
Carc M Gl ♀ X
Un-certain ♀ 43.
Broncho-Pneumonia ♂ 70.
Carc M Gl Metas Lungs & 630. Liver ♀
Carc Lung Metas Mediastinum ♀ 326.
Pul Hem. ♂ 542.
Carc Lung. ♀ 258.
Myo-carditis. ♂ 814.
Sarc[d] Ovary. Metas Liver-Kid. Mesentery ♀ 26.
Sarc[d] Liver Adenoma Lung. ♀ 5743.
Abs M Gl.[d] Liver Abs.[d] ♀ 2429.
Perito-nitis ♀ 86.
Adenoma Liver Pyelo-Neph. ♀ 1681.
Sarc Kidney ♀ 348.
Pul Inf. ♀ 1199.
Pul Inf ♂ 3227.
Adenoma Liver Pap Lung ♂ 11836.
Perito-neal Hem. ♀ 349.
Int Inf. ♂ 742.
Acute Inf ♂ 824.
Carc M Gl. Prim-Carc Lungs ♀ 1052.
Mult Adenomata Liver Prim. Adenoma Lung. ♂ 830.
Unk Inf ♀ 272.
Edema Lungs ♂ 1210.
2 Malig Tumors of Liver ♀ 1453.
Liver Cyst Adenoma Lung ♂ 1845.
Prim. Carc Lung Metas. Lung ♀ 4198.
Uterine Infection ♀ 165.
Uncertain ♂ 23.
Puerperal Infection ♀ 94.
Pseudo-Leukemia ♂ 18.
Carc M Gl Metas Lungs ♀ 12.
Uncertain ♂ 10.
Acute Inf. ♀ 127.
Sq Cell Carc. Skin Carc Cyst Ovary ♀ 496.

CHART 6

*Adenoma, Endothelioma, Papilloma*

*Chart 7*

*Strain 201.* This strain is derived from the same source on the maternal side as Strain 202, female No. 1681 of the third filial generation (shown in Chart 5) being the point of departure from that strain. She died of an adenoma of the liver like that of her great-grandmother. Mated with her brother, male No. 616, she produced female No. 1211 with carcinoma of the mam-

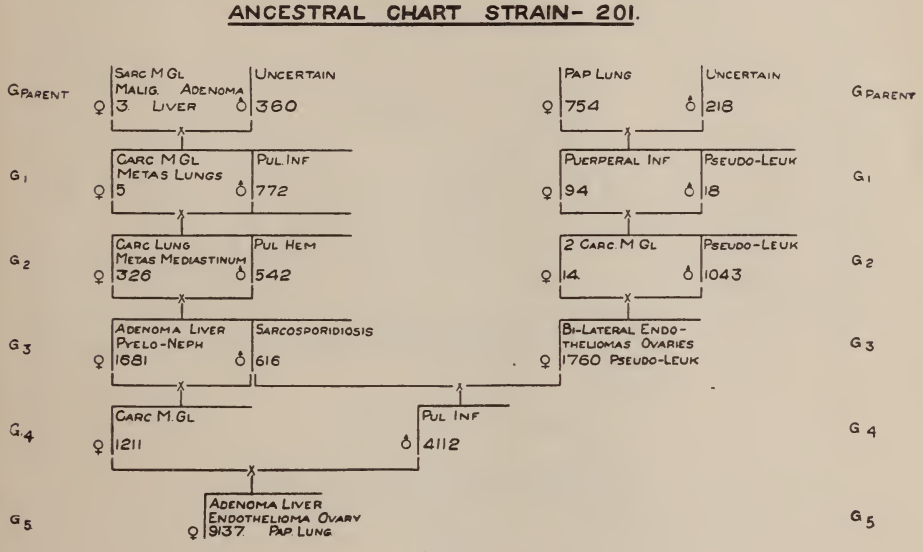



CHART 7

mary gland. This same male No. 616 was mated with female No. 1760 (of Strain 196, Chart 5) who died of bilateral endothelioma of the ovaries and of pseudoleukemia. Their son, male No. 4112, with pulmonary infection, was mated with female No. 1211. The end product of this mating was female No. 9137. She reproduced the adenoma of the liver of her maternal grandmother, female No. 1681, and of her grandmother of many generations earlier, female No. 3; she reproduced also the ovarian endothelioma of her paternal grandmother, female No. 1760, and the papilloma of the lung carried by female No. 754, who

antedated her five generations in direct paternal line. Note also the three successive generations of pseudoleukemia shown in the paternal side of this strain.

### TUMORS OF THE JAW

*Chart 8*

*Strain 104* is a strain of *Peromyscus Californicus* (a species of the wild "white-foot") secured from the Santa Clara Valley,

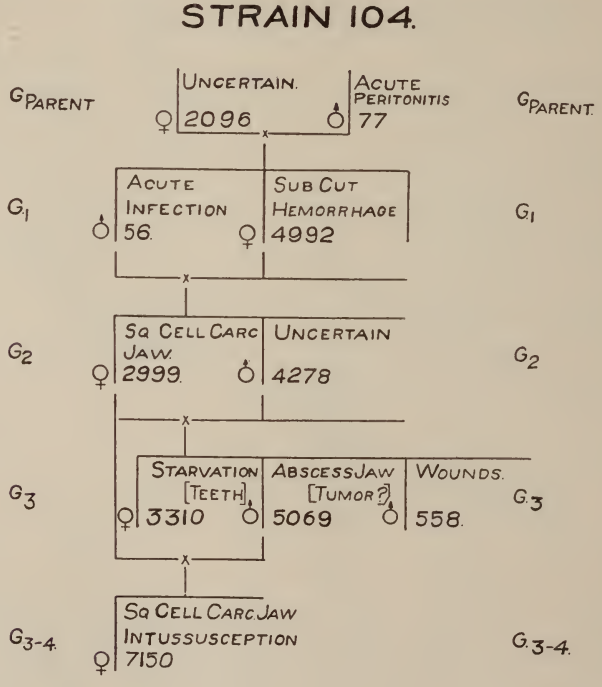



CHART 8

California. In the second generation female No. 2999, with squamous cell carcinoma of the jaw, was mated with her son, male No. 5069, who showed at autopsy a totally necrotic mass upon the jaw. Clinically it behaved like a carcinoma. Since it was totally necrotic, however, when autopsied, it was recorded as an abscess in order to be conservative. Their one offspring

that has died up to date, female No. 7150, showed a squamous cell carcinoma of the jaw.

It is noteworthy that throughout the entire stock of *Peromyscus* in my hands for eight years there has never been another case of squamous cell carcinoma of the jaw.

*Carcinoma, Sarcoma, Pseudoleukemia*[4]

*Chart 9*

*Strain 405* is a hybrid strain derived by crossing Strain 65 (Chart 3), which carried a high percentage of mammary gland carcinoma, with Strain 123. Note the four consecutive generations of carcinoma of the mammary gland in branch C, the appearance of sarcomas and of pseudoleukemia in branch B, and the freedom from tumors of any sort in branch A. This strain also shows pseudoleukemia in three consecutive generations, each succession being represented by uncle and nephew. This chart is a noteworthy example of the apparent segregation into different branches of the same family of types of tumor which entered into the ancestry of the strain.

TUMORS OF UTERUS

*Sarcoma, malignant adenoma*

*Chart 10*

Chart 10 is drawn to show the occurrence of uterine tumors, and all fraternities not immediately concerned are omitted in order to get the data within the limits of a chart.[5]

[4] That leukemia and pseudoleukemia occur in cancer strains has been observed repeatedly in this stock; this fact has also been noted by others working with mouse tumors. In recording this, however, no assumptions are made and no conclusions are drawn concerning the neoplastic nature of leukemia and pseudoleukemia. This relation will receive more extended consideration in the future.

[5] In order to make clear the origin of these strains, at the lower portion of this plate the progenitors of Strain 139 and of Strain 90 are charted with the members of the first filial generation which are concerned in the formation of these strains.

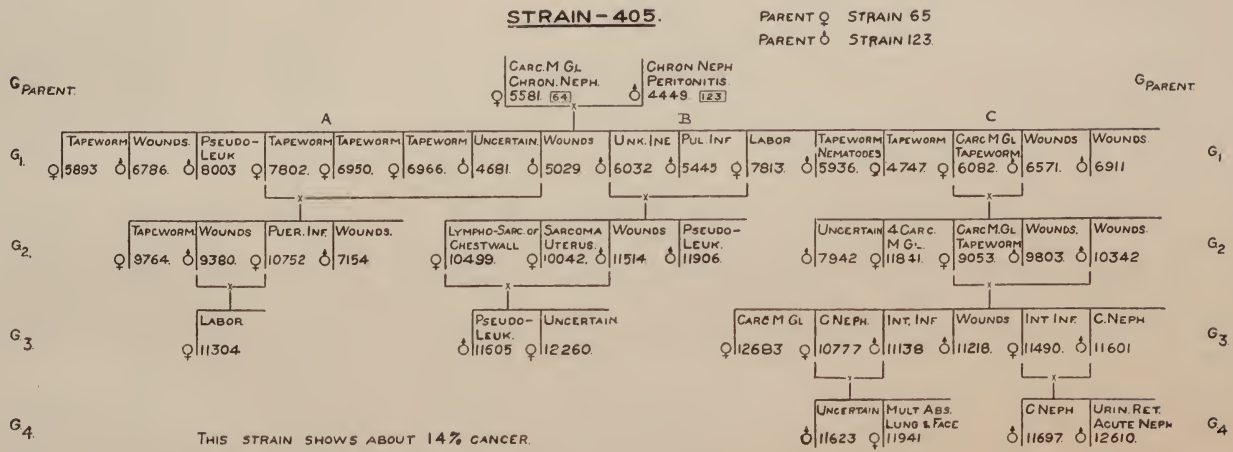
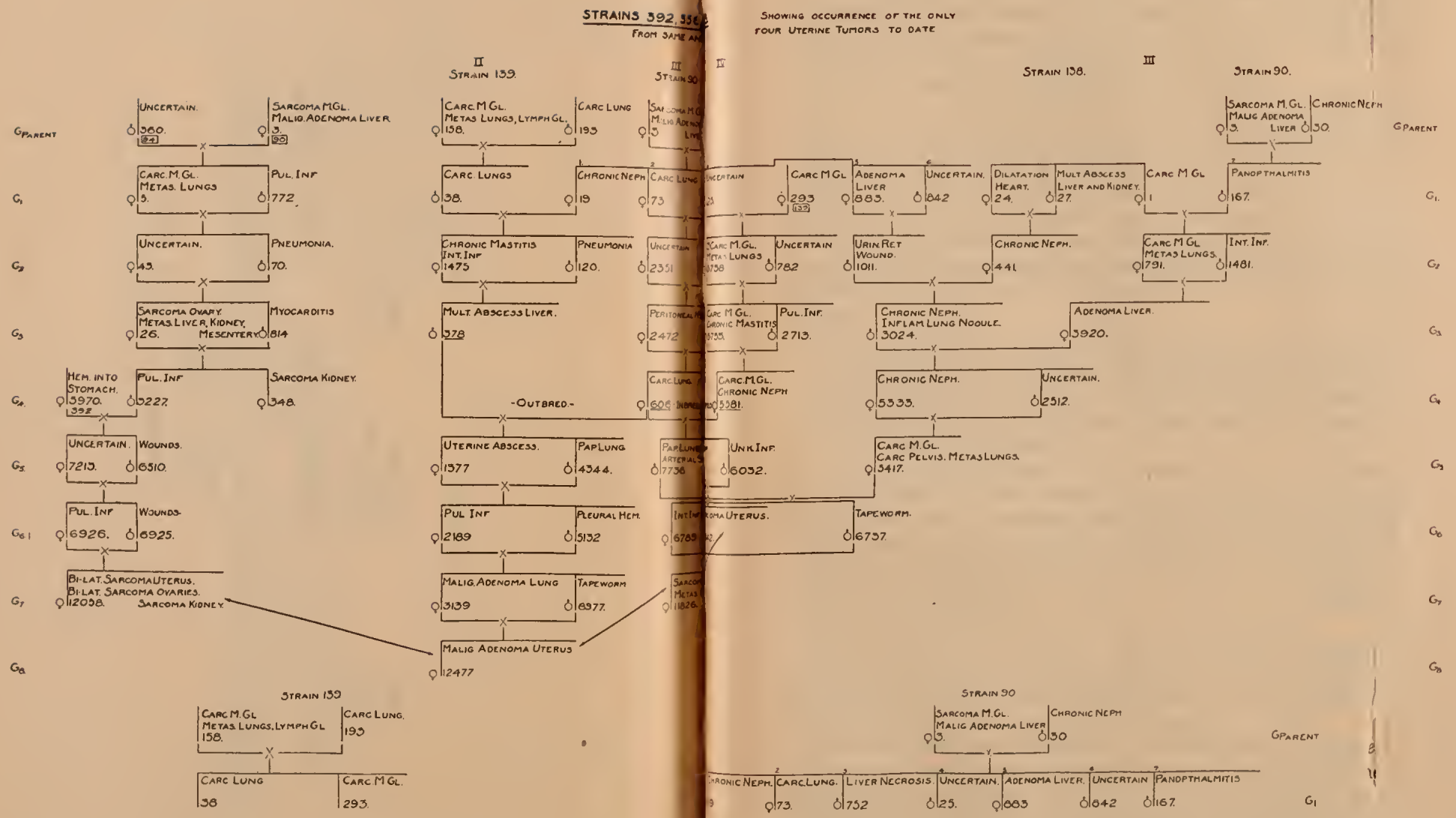

STRAIN-405.
PARENT ♀ STRAIN 65
PARENT ♂ STRAIN 123
G PARENT.
CARC. M GL. CHRON. NEPH. ♀ 5581. 64
CHRON NEPH PERITONITIS. ♂ 4449. 123
G PARENT.
A
B
C
G1.
TAPEWORM ♀ 5893
WOUNDS. ♂ 6786.
PSEUDO-LEUK ♂ 8003
TAPEWORM ♀ 7802.
TAPEWORM ♀ 6950.
TAPEWORM ♀ 6966.
UNCERTAIN. ♂ 4681.
WOUNDS ♂ 5029.
UNK. INF. ♂ 6032
PUL INF ♂ 5445
LABOR ♀ 7813.
TAPEWORM NEMATODES ♂ 5936.
TAPEWORM ♀ 4747.
CARC M GL TAPEWORM ♀ 6082.
WOUNDS ♂ 6571.
WOUNDS. ♂ 6911
G1
G2.
TAPEWORM ♀ 9764.
WOUNDS ♂ 9380.
PUER. INF. ♀ 10752
WOUNDS. ♂ 7154
LYMPHO-SARC. OF CHESTWALL ♀ 10499.
SARCOMA UTERUS. ♀ 10042.
WOUNDS ♂ 11514
PSEUDO-LEUK. ♂ 11906.
UNCERTAIN ♂ 7942
4 CARC. M GL. ♀ 11841.
CARC M GL TAPEWORM ♀ 9053.
WOUNDS. ♂ 9803.
WOUNDS. ♂ 10342
G2
G3.
LABOR ♀ 11304
PSEUDO-LEUK. ♂ 11605
UNCERTAIN ♀ 12260.
CARC M GL ♀ 12683
C NEPH. ♀ 10777
INT. INF ♂ 11138
WOUNDS ♂ 11218.
INT INF. ♀ 11490.
C. NEPH ♂ 11601
G3
G4.
UNCERTAIN ♂ 11623
MULT ABS. LUNG & FACE ♀ 11941
C NEPH ♂ 11697.
URIN. RET. ACUTE NEPH ♂ 12610.
G4
THIS STRAIN SHOWS ABOUT 14% CANCER.

CHART 9


STRAINS 392, 55[illegible]
FROM SAME AN
SHOWING OCCURRENCE OF THE ONLY FOUR UTERINE TUMORS TO DATE
II
STRAIN 139.
III
STRAIN 90.
II
STRAIN 138.
III
STRAIN 90.
GPARENT
G1
G2
G3
G4
G5
G6
G7
G8
UNCERTAIN. ♂360. SARCOMA M.GL. MALIG. ADENOMA LIVER. ♀3.
CARC. M. GL. METAS. LUNGS ♀5. PUL. INF. ♂772.
UNCERTAIN. ♂43. PNEUMONIA. ♂70.
SARCOMA OVARY. METAS. LIVER, KIDNEY, MESENTERY ♀26. MYOCARDITIS ♂814.
HEM. INTO STOMACH. ♀3970. PUL. INF. ♂5227. SARCOMA KIDNEY. ♀348.
UNCERTAIN. ♀7213. WOUNDS. ♂6510.
PUL. INF. ♀6926. WOUNDS. ♂6925.
BI-LAT. SARCOMA UTERUS. BI-LAT. SARCOMA OVARIES. ♀12038. SARCOMA KIDNEY.
CARC. M. GL. METAS. LUNGS, LYMPH GL. ♀158. CARC. LUNG ♂193.
CARC. LUNGS ♂38. CHRONIC NEPH. ♀19.
CHRONIC MASTITIS INT. INF ♀1475. PNEUMONIA ♂120.
MULT. ABSCESS LIVER. ♂378.
-OUTBRED-
UTERINE ABSCESS. ♀1377. PAP. LUNG ♂4344.
PUL. INF. ♀2189. PLEURAL HEM. ♂5132.
MALIG. ADENOMA LUNG ♀3139. TAPEWORM ♂6977.
MALIG. ADENOMA UTERUS ♀12477.
CARC. LUNG ♂73. CARC. M. GL. ♀293.
CARC. M. GL. METAS. LUNGS ♀738. UNCERTAIN ♂782.
PERITONEAL M[illegible] ♀2472. CARC. M. GL. CHRONIC MASTITIS ♂3733. PUL. INF. ♂2713.
CARC. LUNG. ♀6065 INBRED. CARC. M. GL. CHRONIC NEPH ♂5381.
PAP. LUNG ARTERIAL ♀7738. UNK. INF. ♂6032.
INT. INF. ♀6789. TAPEWORM. ♂6737.
UNCERTAIN ♂23. CARC. M. GL. ♀293. ADENOMA LIVER ♂883. UNCERTAIN. ♂842. DILATATION HEART. ♀24. MULT. ABSCESS LIVER AND KIDNEY. ♀27. CARC. M. GL. ♀1. PANOPTHALMITIS ♂167.
URIN. RET. WOUND. ♂1011. CHRONIC NEPH. ♀441. CARC. M. GL. METAS. LUNGS. ♀1791. INT. INF. ♂1481.
CHRONIC NEPH. INFLAM. LUNG NODULE. ♀3024. ADENOMA LIVER. ♂3920.
CHRONIC NEPH. ♀5333. UNCERTAIN. ♂2512.
CARC. M. GL. CARC. PELVIS. METAS. LUNGS. ♀3417.
TAPEWORM. ♂6737.
SARCOMA M. GL. MALIG. ADENOMA LIVER ♀3. CHRONIC NEPH ♂30.
STRAIN 139
CARC. M. GL. METAS. LUNGS, LYMPH GL. ♀158. CARC. LUNG. ♂193.
CARC. LUNG ♂38. CARC. M. GL. ♀293.
STRAIN 90
SARCOMA M. GL. MALIG. ADENOMA LIVER ♀3. CHRONIC NEPH ♂30.
CHRONIC NEPH. ♂9. CARC. LUNG. ♀73. LIVER NECROSIS. ♂752. UNCERTAIN. ♀25. ADENOMA LIVER. ♂883. UNCERTAIN ♂842. PANOPTHALMITIS ♂167.

The genealogy of these uterine tumor mice is here charted back to its source in my hands, so that it may be clear to the reader how inevitably the same sources furnished uterine tumor no matter what the nature of the mating. Let me especially emphasize some of the striking features of Chart 10.

(1) The significant progenitor here is female No. 3 of Strain 90. *Inbred* with her brother, male No. 30 (shown in Lines II, III, and IV) she originated the strains which produced three of the four uterine tumors in this stock. *Outbred* with male No. 360 of Strain 94 she founded the strain (shown in Line I) which produced the only other uterine tumor which has occurred in the entire twenty-five hundred neoplasms occurring in this laboratory, namely, female No. 12058, with bilateral sarcoma of the uterus, bilateral sarcomas of the ovaries, and a sarcoma of the kidney.

(2) Female No. 3, or her immediate offspring, furnished one or both parents in every line in the somewhat intricate hybridization which led to the sudden outcropping within two thousand autopsies of the only four uterine tumors which have arisen in the entire stock in six years.

(3) In Lines II, III, and IV, filial generations 3 and 4, female No. 606, *inbred* with her brother, male No. 4449, produced the strain which three generations later furnished female No. 11826 with a sarcoma of the uterus and metastasis in the diaphragm. *Outbred* with male No. 378 she headed the strain which four generations later produced female No. 12477, with a malignant adenoma of the uterus.

Her brother, male No. 4449, *inbred* with herself, produced uterine tumor female No. 11826. *Outbred* with female No. 5581, he headed the strain which two generations later produced female No. 10042 with sarcoma of the uterus.

Note then that, first, female No. 3, *whether inbred or outbred*, originated a strain which produced uterine tumor; second, her later direct descendants, female No. 606 and male No. 4449, *both in inbreeding and in hybridization* headed families which produced uterine tumor. No more striking evidence is possible.

Note also the three generations of kidney sarcoma in Line I,

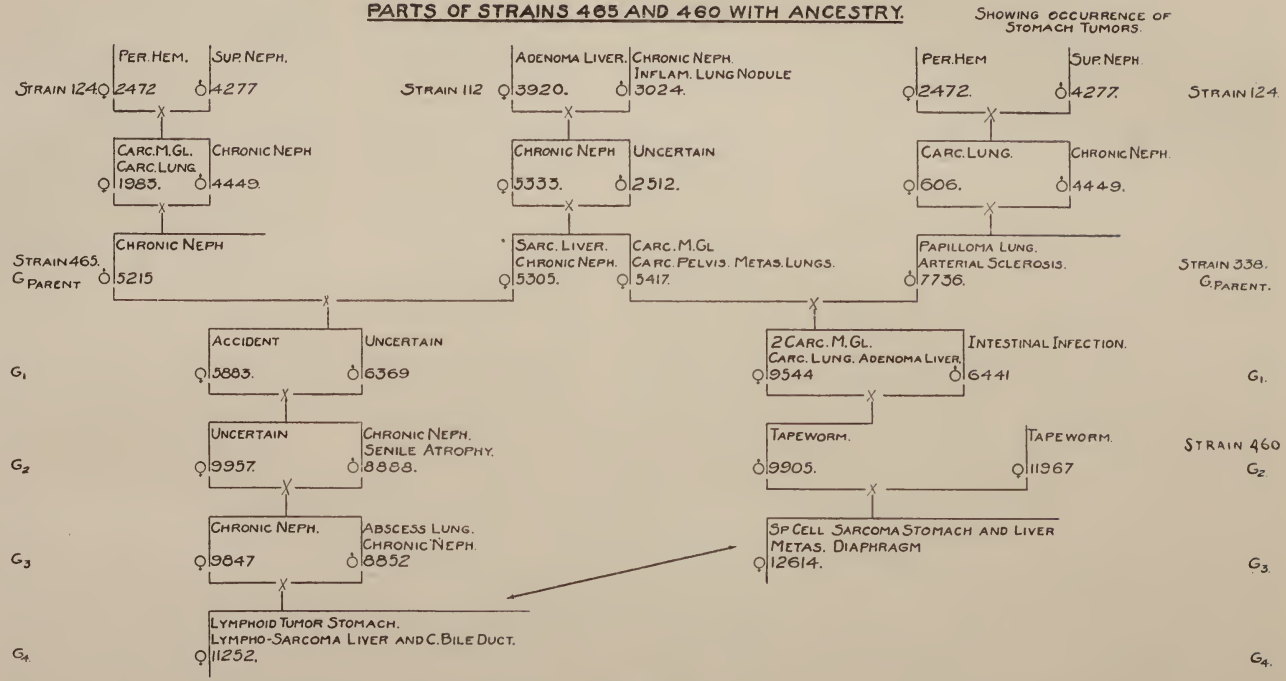

PARTS OF STRAINS 465 AND 460 WITH ANCESTRY.
SHOWING OCCURRENCE OF STOMACH TUMORS.
STRAIN 124 ♀ 2472 PER. HEM. ♂ 4277 SUP. NEPH.
STRAIN 112 ♀ 3920. ADENOMA LIVER. ♂ 3024. CHRONIC NEPH. INFLAM. LUNG NODULE
♀ 2472. PER. HEM ♂ 4277. SUP. NEPH. STRAIN 124.
♀ 1983. CARC. M. GL. CARC. LUNG. ♂ 4449. CHRONIC NEPH
♀ 5333. CHRONIC NEPH ♂ 2512. UNCERTAIN
♀ 606. CARC. LUNG. ♂ 4449. CHRONIC NEPH.
STRAIN 465. G PARENT ♂ 5215 CHRONIC NEPH
♀ 5305. SARC. LIVER. CHRONIC NEPH. ♀ 5417. CARC. M. GL. CARC. PELVIS. METAS. LUNGS.
♂ 7736. PAPILLOMA LUNG. ARTERIAL SCLEROSIS. STRAIN 338. G. PARENT.
G1 ♀ 5883. ACCIDENT ♂ 6369 UNCERTAIN
♀ 9544 2 CARC. M. GL. CARC. LUNG. ADENOMA LIVER. ♂ 6441 INTESTINAL INFECTION. G1.
G2 ♀ 9957. UNCERTAIN ♂ 8888. CHRONIC NEPH. SENILE ATROPHY.
♂ 9905. TAPEWORM. ♀ 11967 TAPEWORM. STRAIN 460 G2
G3 ♀ 9847 CHRONIC NEPH. ♂ 8852 ABSCESS LUNG. CHRONIC NEPH.
♀ 12614. SP CELL SARCOMA STOMACH AND LIVER METAS. DIAPHRAGM G3.
G4 ♀ 11252. LYMPHOID TUMOR STOMACH. LYMPHO-SARCOMA LIVER AND C. BILE DUCT. G4.

CHART 11

and the spotting with adenomas of the liver throughout those portions of the chart derived from female No. 3. Note the dominance of lung tumor in Line II where lung tumor mice started the line, and this even when many fraternities have been omitted which showed kidney tumors, or lung tumors, or liver tumors, as the case may be.

## TUMORS OF THE STOMACH

### *Sarcoma, carcinoma*

### *Chart 11*

The evidence furnished by the stomach tumors is equally striking. There is the same number of stomach tumors in this stock as of uterine tumors, namely, only four, two of them occurring in females and two in males. Charts 11 and 12 are drawn to show the occurrence of stomach tumors, the fraternities not immediately concerned being omitted. It will be noted that Chart 11 includes a part of the same strains shown in Chart 10 and carries identical ancestry.

The striking feature of this chart is as follows: two sisters, females No. 5305 and No. 5417, were mated with two half brothers, males No. 7736 and No. 5215. Each family furnished in the third or in the fourth generation a tumor of the stomach, female No. 12614 with sarcomas of the stomach and liver, and female No. 11252 with a lymphoid tumor of the stomach, and a lympho-sarcoma of the liver and the common bile duct.

The original progenitors of these strains with their intermediate genealogy are shown in Chart 10.

### *Chart 12*

Chart 12 shows the incidence of the other two stomach tumors, which fell in strains closely allied with those shown in Chart 11. The stomach tumors, which are both carcinomas, occur in males No. 7851 and No. 5802, the succession being in uncle and nephew.

## PARTS OF STRAINS 73 AND 384.

SHOWING OCCURRENCE OF STOMACH CARCINOMA

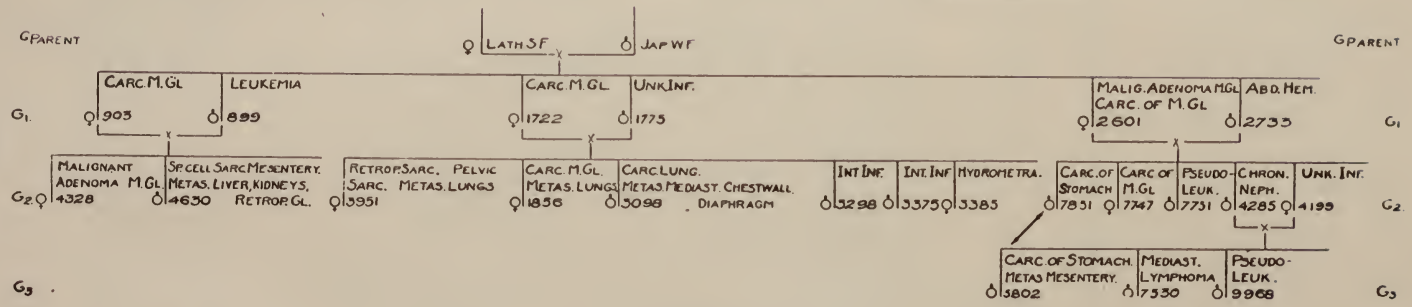



CHART 12

*Chart 13*

*Strain 164.* This strain (previously published in part in the Third Report) shows in both Branch III and Branch IV a predominance of sarcoma in the second hybrid generation with the appearance of carcinoma in the third hybrid generation. Branch IV of this strain (Chart 14) shows in family A one case each of sarcoma and of carcinoma; in family B, one case each of sarcoma, carcinoma and pseudoleukemia; in family C three generations each of sarcoma and of carcinoma; in family D no tumor of any sort.

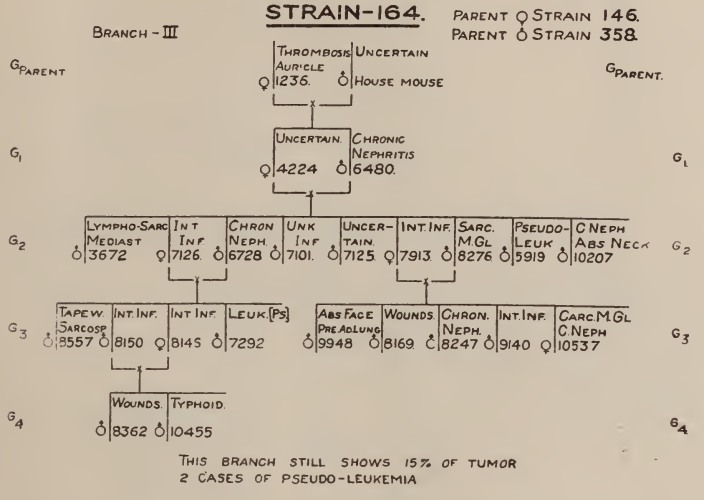



CHART 13

*Chart 14*[6]

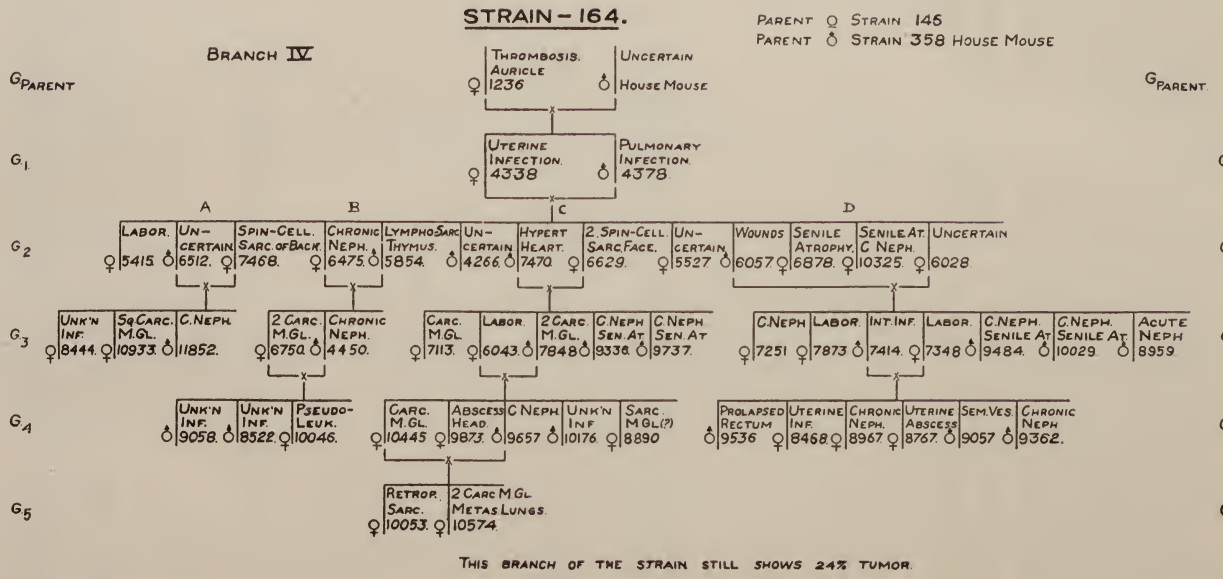
TUMORS OF THE BACK AND SIDES

*Sarcoma*

*Chart 15*

*Strain 186.* This is a strain of closely inbred house-mice in my hands for many years. The tumor carried in this strain is of a type peculiar to the strain. It is of the desmoid type and is

[6] On p. 500.




THIS BRANCH OF THE STRAIN STILL SHOWS 24% TUMOR.

CHART 14

very slow-growing. Throughout the entire twenty-five hundred neoplasms shown in this stock there has been only one other tumor of this type. This also was in a house-mouse. This tumor also runs a clinical course peculiar to itself. It begins with a baldness which invariably appears on the dorsal posterior portion of the body, that portion bitten by the males in fighting one another, and grasped by the male in copulation. In every case observed it has originated on a basis of light, often-repeated scratches and scars. The baldness first occurs at the position of these slight wounds but spreads gradually over

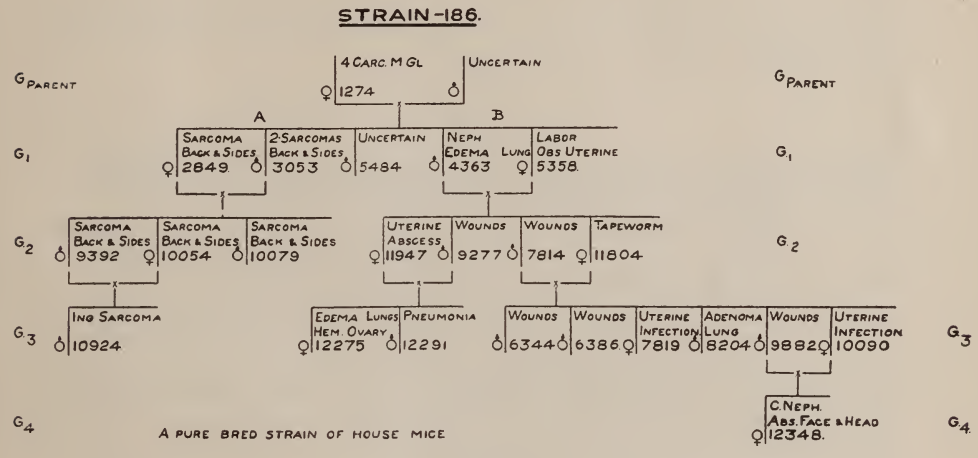



CHART 15

adjacent and entirely unscarred areas, until the entire posterior half of the body is nude of hair. During this process a very slowly progressive induration takes place throughout the bald area. The process advances as a deepening rather than as a broadening induration. The entire posterior half of the body gradually becomes so stiffened by this growth that the mouse can scarcely move, and the immediate cause of death is generally the inability to move to food and water. The growth in male No. 10924 was less widespread than in any of the others. Branch A shows 100 per cent of this tumor, where both parents had it. Branch B where neither parent had tumor, shows no

tumor of any sort until the third filial generation in which there was a small benign adenoma of the lung in female No. 8204. Another remarkable illustration of the persistence of a particular type of tumor within a strain.

The results, then, thus far obtained in this laboratory, basing the conclusions on over fourteen thousand autopsies and on over twenty-five hundred primary neoplasms show, (1) that in every case (not merely in selected cases) where there are sufficient data for study, tumors of specific organs and of specific types persistently occur in strains whose ancestry has furnished that type of tumor, and (2) they rarely or never occur in strains whose ancestry has not furnished that type of tumor, even where the strain carries 100 per cent of cancer.

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(2) The incidence and inheritability of spontaneous cancer in mice (Second Report). The Journal of Medical Research, xxx, no. 3, July, 1914.
(3) The incidence and inheritability of spontaneous cancer in mice (Third Report). The Journal of Medical Research, xxxii, no. 1, March, 1915.
(4) Spontaneous primary tumors of the lung in mice (Fourth Report). Journal of Medical Research, xxx, no. 3, July, 1914.
(5) Spontaneous primary tumors of the liver in mice (Sixth Report). Journal of Medical Research, xxxii, November, 1915.
(6) Third Scientific Report, Imp. Cancer Research Fund, 1908, p. 69.

# THE INHERITABILITY OF SPONTANEOUS TUMORS OF THE LIVER IN MICE[1]

## STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

### Seventh Report

MAUD SLYE

*From the Cancer Laboratory of the Otho S. A. Sprague Memorial Institute and the University of Chicago*

Received for publication, September 18, 1916

The work of the past six years in this laboratory has definitely established two points in regard to the behavior of spontaneous cancer.

### I. CANCER IN GENERAL IS INHERITABLE[2]

This statement is based upon the following observations:

(1) There are strains of mice in this laboratory which under no provocation have ever been made to produce spontaneous cancer.

(2) There are other strains of mice which, under the right provocation, inevitably do produce cancer.

(3) It is possible by selective breeding to manipulate these two types of mouse strains with the same certainty with which it is possible to manipulate pure-breeding pigmented mice and pure-breeding albinos, and to derive therefrom pure-breeding cancer strains and pure-breeding non-cancer strains.

I say "cancer in general," because until one has completely

[1] Presented before the American Association for Cancer Research, Washington, D. C., May, 1916.

[2] For the sake of brevity I shall use the expression "inheritability of cancer," although cancer is not transmitted as such; rather it is transmitted as a tumor-producing potentiality. See introduction to Third Report, Jour. Med. Research, 1915, xxxii, 159.

analyzed the reproductive potentialities of every individual concerned in the genealogy of a family, it is impossible to direct what shall be produced in any given case; and the resulting strain is likely to show sarcomas or carcinomas, adenomas or endotheliomas—of the ovaries, or of the mammary gland, or of the lung, or of the liver, or of any other organ. A family, therefore, manufactured by the hybridization of cancer-bearing with non-cancer-bearing individuals, will be peppered with tumors of different organs and of different types. Even after the cancer-bearing potentialities of two parents have been analyzed as completely as possible, we still discover in the offspring some divergence from the ancestral type. From such experiments carried on through years and in enormous numbers, yielding year after year perfectly consistent and logical results, no matter what strains are used, there is but one conclusion possible, namely, that this tumor-producing potentiality is a thing indubitably transmitted by the right selective breeding.

## II. TUMORS OF SPECIFIC ORGANS AND OF SPECIFIC TYPES ARE INHERITABLE

That is, by selective breeding it is possible to derive strains of mice which yield a high percentage of lung tumor or of mammary gland tumor, or of liver tumor, or even of stomach and uterine tumors which are very rare in mice. And these strains of mice, again, can be manipulated, carrying into strains with which they are hybridized the types of tumors borne in their own ancestral line but from which the family with which they are crossed is perfectly free. A general survey of this field appears in the fifth report of these studies published in this issue.

The present report concerns itself with the inheritability of a single class of these tumors of specific organs, namely, tumors of the liver.

The data presented by a single strain or by many strains carrying a high percentage of mammary gland or of lung tumors when inbred, and introducing a high percentage of tumors of

these same organs into every strain with which they are hybridized, might be contested on the ground of the frequency of these types of spontaneous tumors in mice, since about 90 per cent of all reported mouse tumors occur in these organs. Or the demonstration of a relatively high frequency of stomach or of uterine tumors in certain strains might fail to convince, because of the paucity of these tumors in mice.

But tumors of the liver are of sufficient rarity in the literature to make an interesting study and are sufficiently frequent in this stock to make a most striking one. In all mouse literature outside of these studies there is but one tumor of the liver reported, namely, an adenoma reported by the Imperial Cancer Research Fund of London (Third Report).

This stock to date has furnished sixty-two primary tumors of the liver. These tumors are chiefly adenomas, with a sprinkling of sarcomas, and of carcinomas, and they are described fully in the Fourth Report of this series.[3] It has furnished seventeen secondary tumors of the liver; these are mostly spindle-cell and osteosarcomas, with a few carcinomas. All these primary liver tumors have fallen in strains of identical ancestry, namely, ancestry derived from Strain 90, and among all the other cancer-bearing strains, yielding twenty-five hundred primary neoplasms appearing in this stock, not one liver tumor has occurred. This fact furnishes a most striking piece of negative evidence for the inheritability of liver tumors.

*Chart 1*

*Strains 280 and 281.* These strains were produced by mating female No. 258, who died of a carcinoma of the lung but who came of a family carrying a heavy percentage of liver tumors (see Chart 8, Strain 202), with two brothers from Strain 90, namely, male No. 363 and male No. 286.

1. Mated with male No. 363, who also had carcinoma of the lung, she produced a line of which 100 per cent carry tumor, 80 per cent of these tumors being tumor of the lungs.

[3] Jour. Med. Research, 1915, xxxii, 171.

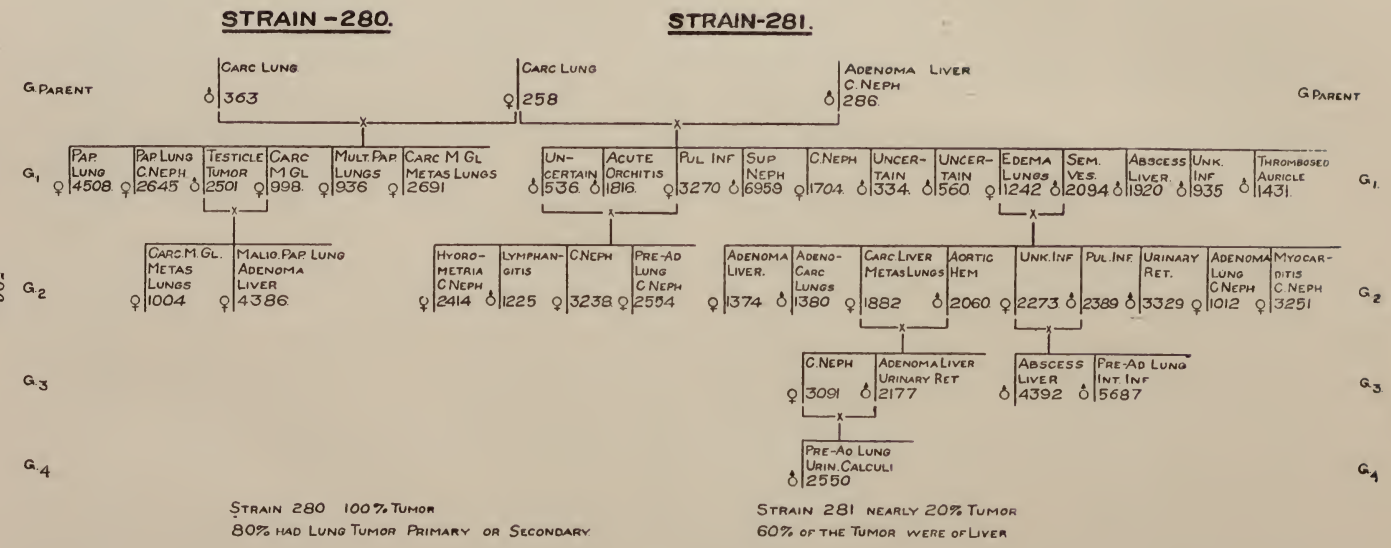

STRAIN -280.
STRAIN-281.
G. PARENT
CARC LUNG ♂ 363
CARC LUNG ♀ 258
ADENOMA LIVER C. NEPH ♂ 286
G. PARENT
G1
PAP. LUNG ♀ 4508
PAP LUNG C NEPH ♂ 2645
TESTICLE TUMOR ♀ 2501
CARC M GL ♀ 998
MULT. PAP LUNGS ♀ 936
CARC M GL METAS LUNGS ♀ 2691
UN-CERTAIN ♂ 536
ACUTE ORCHITIS ♂ 1816
PUL. INF ♀ 3270
SUP NEPH ♂ 6959
C. NEPH ♀ 1704
UNCER-TAIN ♂ 334
UNCER-TAIN ♂ 560
EDEMA LUNGS ♀ 1242
SEM. VES. ♂ 2094
ABSCESS LIVER ♂ 1920
UNK. INF ♂ 935
THROMBOSED AURICLE ♂ 1431
G1
G2
CARC M. GL. METAS LUNGS ♀ 1004
MALIG. PAP. LUNG ADENOMA LIVER ♀ 4386
HYDRO-METRIA C NEPH ♀ 2414
LYMPHAN-GITIS ♂ 1225
C. NEPH ♀ 3238
PRE-AD LUNG C NEPH ♀ 2554
ADENOMA LIVER. ♀ 1374
ADENO-CARC LUNGS ♂ 1380
CARC LIVER METAS LUNGS ♀ 1882
AORTIC HEM ♂ 2060
UNK. INF ♀ 2273
PUL. INF ♂ 2389
URINARY RET. ♂ 3329
ADENOMA LUNG C NEPH ♀ 1012
MYOCAR-DITIS C. NEPH ♀ 3251
G2
G3
C. NEPH ♀ 3091
ADENOMA LIVER URINARY RET ♂ 2177
ABSCESS LIVER ♂ 4392
PRE-AD LUNG INT. INF ♂ 5687
G3
G4
PRE-AD LUNG URIN. CALCULI ♂ 2550
G4
STRAIN 280 100% TUMOR
80% HAD LUNG TUMOR PRIMARY OR SECONDARY
STRAIN 281 NEARLY 20% TUMOR
60% OF THE TUMOR WERE OF LIVER

CHART 1

2. Mated with male No. 286 with an adenoma of the liver, she produced a line showing three cases of liver tumor in two generations, or 9 per cent of liver tumor. There was in this strain also an adenocarcinoma of the lungs, an adenoma of the lungs, and three lung nodules showing pre-adenomatous hyperplasia.

*Chart 2*

*Strains 245 and 246.* These strains were produced by mating male No. 286 (parent male also in Chart 1), with an adenoma of the liver, with two sisters of Strain 48. Mated with female No. 412, who died of acute nephritis, he produced a strain showing over 44 per cent of tumor, with four straight generations of carcinoma of the mammary gland, three generations of adenoma of the lung, and one case of "fibro-endothelioma" of the ovary. Mated with the latter's sister, female No. 26, with sarcoma of the ovary, liver, kidney and mesentery,[4] he produced a line showing tumor in 46 per cent of the individuals, and giving two generations of primary liver tumors.

Note then, that in both instances where male No. 286 was mated with a female having a liver tumor, the resulting offspring showed a high percentage of liver tumor. When the same male was mated with a female without liver tumor no liver tumor appeared in the resulting strain although that strain carried a high percentage of tumor.

*Chart 3*

*Strain 202.* This chart is drawn to show both maternal and paternal ancestry, giving only the fraternities which are concerned in the production of liver tumors. The individuals of this strain and its ancestry and of its sister strain, Strain 48 (inbred) show nine cases of liver tumor among thirty-eight individuals, or liver tumor in nearly 24 per cent of the individuals; a tremendous percentage, considering the rarity of these tumors

[4] It is practically impossible in this case to say which of these sarcomas is primary but it is probable that the ovarian tumor is primary; hence the liver, kidney and mesenteric tumors have been classed as secondary.

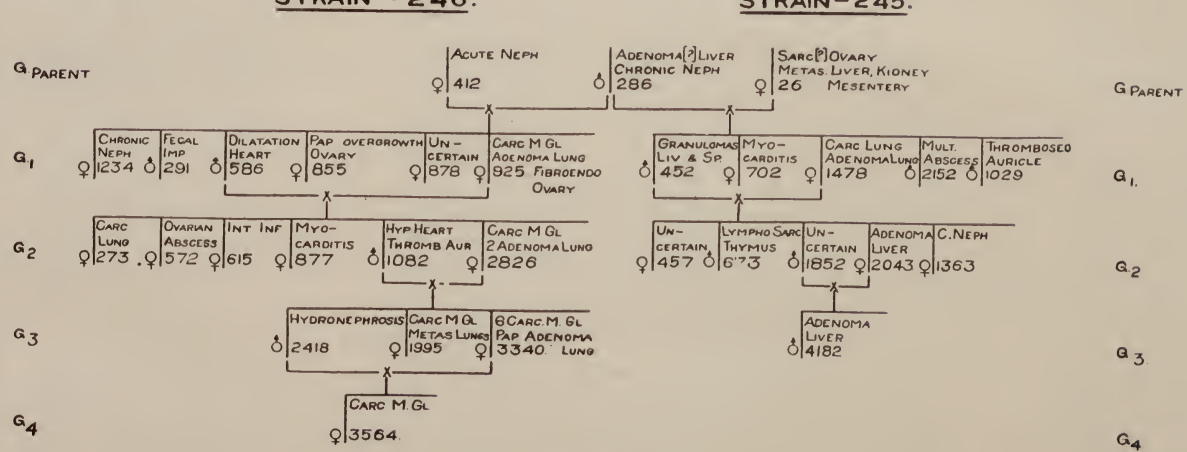

STRAIN – 246.
STRAIN – 245.
G PARENT
Acute Neph ♀ 412
Adenoma [?] Liver Chronic Neph ♂ 286
Sarc [P] Ovary Metas. Liver, Kidney ♀ 26 Mesentery
G PARENT
G1
Chronic Neph ♀ 1234
Fecal Imp ♂ 291
Dilatation Heart ♂ 586
Pap overgrowth Ovary ♀ 855
Un – certain ♀ 878
Carc M Gl Adenoma Lung ♀ 925 Fibroendo Ovary
Granulomas Liv & Sp ♂ 452
Myo – carditis ♀ 702
Carc Lung Adenoma Lung ♀ 1478
Mult. Abscess ♂ 2152
Thromboses Auricle ♂ 1029
G1
G2
Carc Lung ♀ 273
Ovarian Abscess ♀ 572
Int Inf ♀ 615
Myo – carditis ♂ 877
Hyp Heart Thromb Aur ♂ 1082
Carc M Gl 2 Adenoma Lung ♀ 2826
Un – certain ♀ 457
Lympho Sarc Thymus ♂ 6773
Un – certain ♀ 1852
Adenoma Liver ♀ 2043
C. Neph ♀ 1363
G2
G3
Hydronephrosis ♂ 2418
Carc M Gl Metas Lungs ♀ 1995
6 Carc M Gl Pap Adenoma Lung ♀ 3340
Adenoma Liver ♂ 4182
G3
G4
Carc M Gl ♀ 3564
G4

CHART 2

MATERNAL ANCESTRY.

PARENT ♀ STRAIN 90.
PARENT ♂ STRAIN 94

# STRAIN – 202.

PATERNAL ANCESTRY.

PARENT ♀ STRAIN 94
PARENT ♂ STRAIN 90

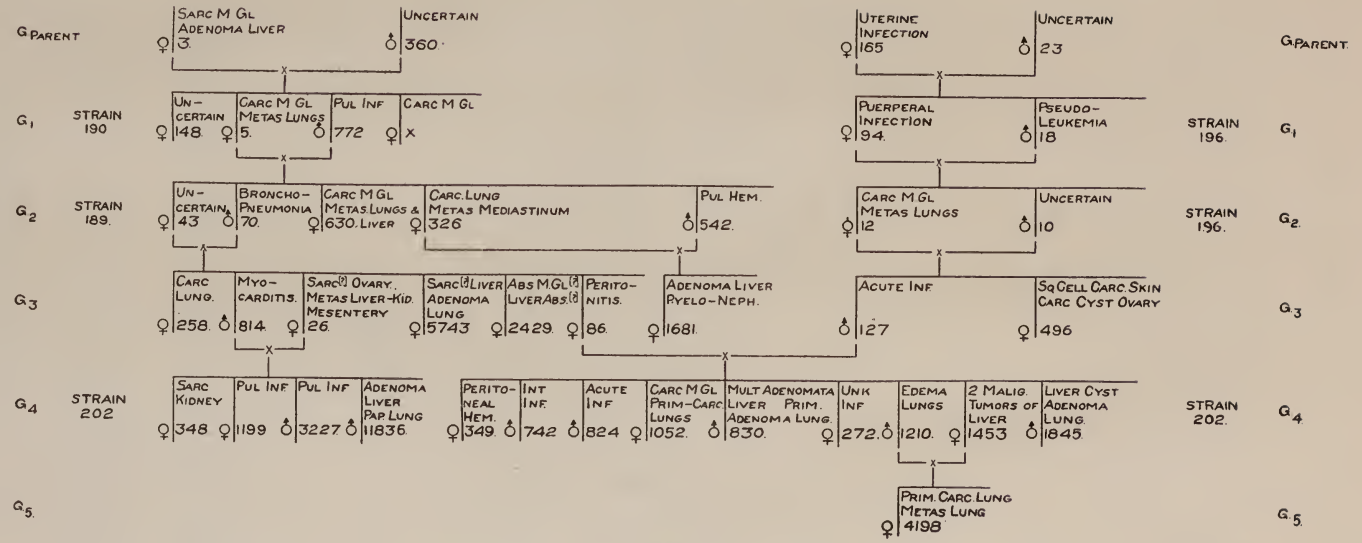



CHART 3

in mice. The construction of this strain involved the use of maternal and paternal ancestry from identical stock, carrying liver tumor. The potency of female No. 3 to transmit liver tumor is shown in strain after strain derived from her.

Note here also that female No. 26 when *inbred* with her brother, male No. 814, with myocarditis, produced offspring with liver tumor, just as she did when *hybridized* with male No. 286 in Strain 245 (Chart 2). Note then: Female No. 258 (Chart 1) with *lung carcinoma* and coming from *a strain rich in liver tumor* mated with a male with *lung carcinoma* produced *80 per cent of lung tumor;* the same female mated with male No. 286 with *adenoma of the liver* produced *9 per cent of liver tumor.*

Again, male No. 286 with *adenoma of the liver* mated with female No. 258, *produced liver tumor;* mated with female No. 26 of Strain 48 (Chart 3), *he produced liver tumor,* while mated with female 412 without liver tumor and coming from a branch of the family *rich in mammary gland tumor,* he produced *four generations of mammary gland tumor.*

Again, female 26 with sarcoma of the liver, *outbred* as above with male No. 286, with adenoma of the liver, produced liver tumor. *Inbred* with male No. 814, she produced liver tumor. The assumption of a chance determinant here is absurd where every individual parent is doubly or trebly checked as to his potentialities in the matter of liver tumor transmission.

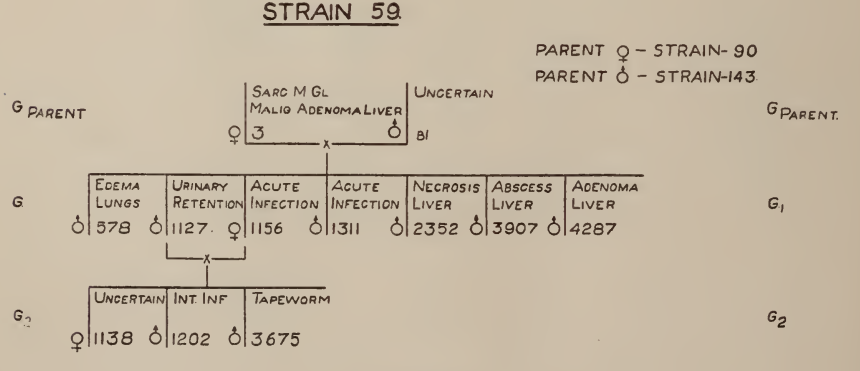



CHART 4

*Chart 4*

*Strain 59.* This is another strain derived from female No. 3, crossed with a male from Strain 143 who died before autopsies were being made. Here again she produced offspring with adenoma of the liver. Strain 143 never showed liver tumor.

*Chart 5*

*Strain 215.* The parent of this strain, female No. 630, with metastatic carcinoma of the liver, is shown with her ancestry in Chart 3, second filial generation. She was crossed with male No. 721 of an allied line to make Strain 215. The resulting strain shows three cases of primary liver tumor in three successive generations.

*Chart 6*

Chart 6 shows a portion of Strain 215 with its parentage for six generations. The result is seven straight generations of tumor, three of these involving primary tumor of the liver.

We come now to the consideration of a strain which alone has produced twelve cases of primary liver tumor, namely, Strain 338. It is a strain manufactured during the last four years from stock deliberately selected to test the inheritability of tumor types, with emphasis upon liver tumors.

1. It must be remembered that in work with mice, liver tumor can not be diagnosed until autopsy. In deliberately breeding to test the inheritability of liver tumor therefore, one is blind as to whether or not the individual selected will show it.

2. It must be remembered again that in order to get sufficient numbers of offspring from the tested individuals for such a rare tumor as liver tumor, the selected mice must breed young. One must therefore frequently use mice which have not yet shown any tumor at all.

3. The worker, then, must be guided by the ancestral history, and by the available data in the fraternities of the individuals selected for this test.

In spite of all these handicaps note the results:

## STRAIN-215.

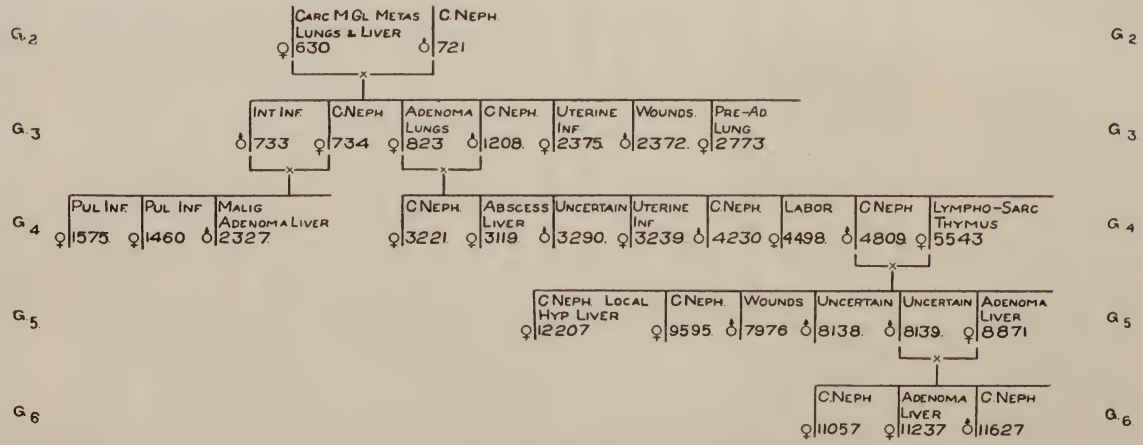



Chart 5

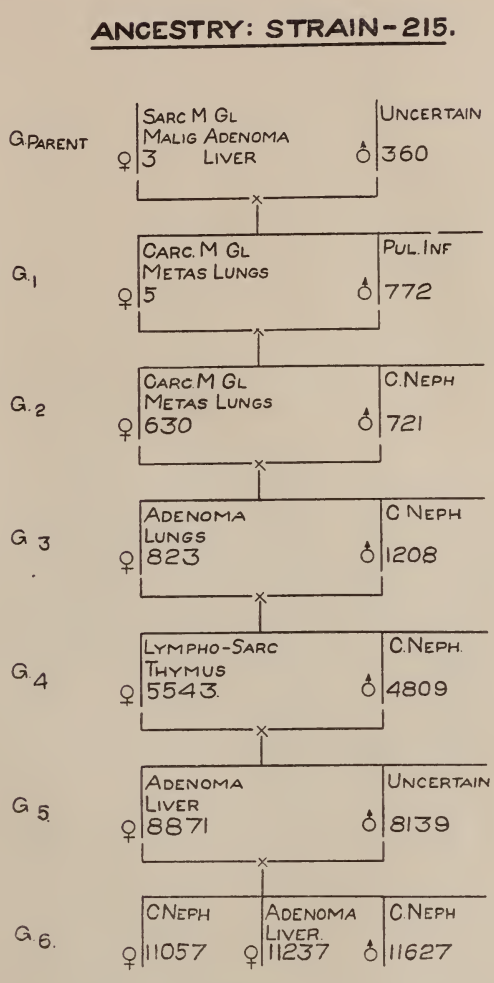



CHART 6

*Chart 7*

*Strain 112.* The parent female of this liver tumor strain, Strain 338, was female No. 5417. She presented no evidence of liver tumor but was selected to start Strain 338 after the appearance of her mammary gland carcinoma. She had six litters of young after the appearance of her tumor and is one of the individuals previously reported (Third Report, p. 193), in whom tumor growth

## STRAIN-112

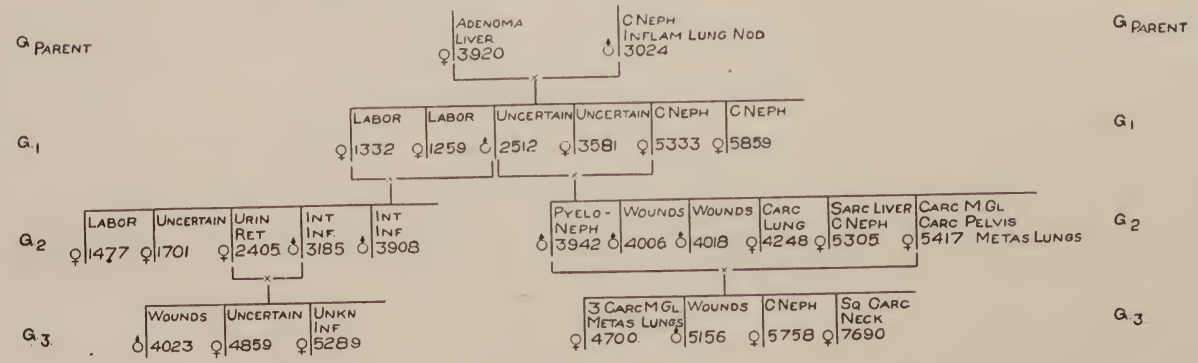



CHART 7

was greatly retarded by constant pregnancy. She lived nearly a year after the appearance of her mammary gland carcinoma and showed at autopsy an additional carcinoma of the pelvis and lungs riddled with metastases. Indeed, at the time of her death the mouse was about one-half carcinoma.

The place of this female within her inbred strain is shown in Chart 7, second filial generation. It will be noted that her grandmother, female No. 3920, had adenoma of the liver, and that her sister born in the same litter, female No. 5305, had sarcoma of the liver. Inbred with her brother, male No. 3942, with pyelo-nephritis, she produced mammary gland and neck carcinoma with metastases in the lungs.

*Chart 8*

*Strain 124.* The paternal parent of Strain 338 was male No. 7736. He was selected as the strongest remaining representative of strain 124, which had liver tumor ancestry behind it. His great-aunt, female No. 1070, had adenoma of the liver. His immediate family at the time he was selected showed 25 per cent of cancer, but no liver tumor.

He himself lived to be three years and three months old. He died of arterial sclerosis, and showed at autopsy a papilloma of the lung.

His place in his inbred strain is shown in Chart 8, Branch II, filial generation 3.

*Chart 9*

Chart 9 is drawn to show the complete ancestry which lay behind these two parents, namely, female No. 5417 and male No. 7736.

*Ancestry of female No. 5417.* Five generations back her grandparents were female No. 3, with a malignant adenoma of the liver, inbred with her brother, male No. 30. with chronic nephritis (these original forbears were members of Strain 90).

The male in her maternal line, and the female in her paternal line, were the immediate offspring of this pair, the female No.

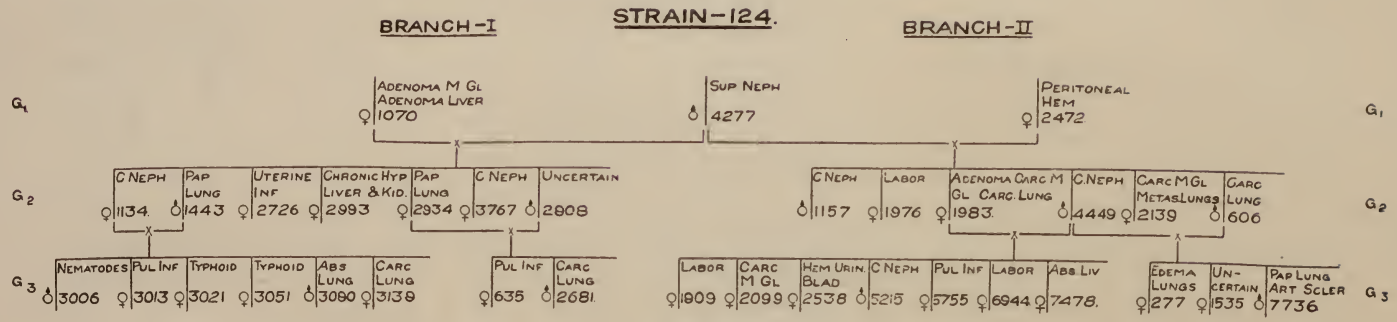
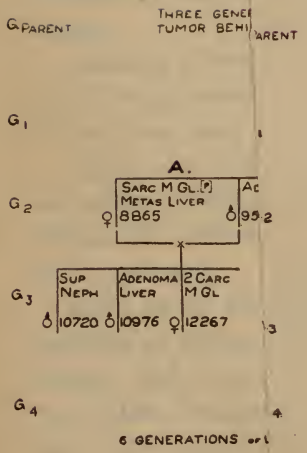

BRANCH-I
STRAIN-124.
BRANCH-II
G1
Adenoma M Gl
Adenoma Liver
♀1070
Sup Neph
♂4277
Peritoneal
Hem
♀2472
G1
G2
C Neph
♀1134
Pap
Lung
♂1443
Uterine
Inf
♀2726
Chronic Hyp
Liver & Kid.
♀2993
Pap
Lung
♀2934
C Neph
♀3767
Uncertain
♂2908
C Neph
♂1157
Labor
♀1976
Adenoma Carc M
Gl Carc Lung
♀1983
C Neph
♂4449
Carc M Gl
Metas Lungs
♀2139
Carc
Lung
♂606
G2
G3
Nematodes
♂3006
Pul Inf
♀3013
Typhoid
♀3021
Typhoid
♀3051
Abs
Lung
♂3080
Carc
Lung
♀3139
Pul Inf
♀635
Carc
Lung
♂2681
Labor
♀1909
Carc
M Gl
♀2099
Hem Urin
Blad
♀2538
C Neph
♂5215
Pul Inf
♀5755
Labor
♀6944
Abs Liv
♀7478.
Edema
Lungs
♀277
Un-
certain
♀1535
Pap Lung
Art Scler
♂7736
G3

CHART 8


G PARENT
THREE GENE
TUMOR BEHI
ARENT
G1
A.
G2
SARC M GL.
METAS LIVER
8865
95.2
G3
SUP
NEPH
10720
ADENOMA
LIVER
10976
2 CARC
M GL
12267
G4
6 GENERATIONS

883 having adenoma of the liver. Hybridization in this generation was with Granby, Mass., red stock, namely, female No. 1, female No. 24 and male No. 27, which never in my hands produced liver tumor, primary or secondary, either in inbreeding or in hybridization with non-liver tumor strains. Female No. 1, however, carried carcinoma, and male No. 27 had abscess of the liver.

The parent female two generations back, female No. 3920, had adenoma of the liver and the male, No. 3024, had an infected lung nodule and chronic nephritis.

There were, then, behind the female, three generations of liver tumor and an ancestry carrying a considerable percentage of cancer, with a tendency of the liver to yield to disease. The origin of both branches of the ancestry was female No. 3 with an adenoma of the liver which appeared later in both sides of the family. These facts determined the selection of female No. 5417, who at the time of her selection had a very small carcinoma of the mammary gland and was in excellent breeding shape.

*Ancestry of male No. 7736.* The original forebears of this line also five generations back were female No. 3 with her adenoma of the liver, and her brother, male No. 30, both long lived.

Again, on both sides of this line also, a double dose of the immediate offspring of female No. 3 was introduced, namely, female No. 73 with carcinoma of the lung and male No. 752 with liver necrosis.

Here as in the maternal line there was a considerable percentage of tumor, a reappearance of adenoma of the liver in the strain, and a tendency of the liver to yield to disease. There were then two generations of liver tumor behind male No. 7736, and three generations of liver tumor behind female No. 5417. Two adenomas of the liver appeared in the first filial generation from this cross, namely, female No. 9544 and male No. 8751.

The rest of the chart shows Branch V, from this cross, bred out in five lines, the female parent of the branch, female No. 8619, showing two carcinomas of the mammary gland, and the male parent of the branch, male No. 8751, an adenoma of the liver.

In the second filial generation there were three tumors of the liver, namely, one sarcoma and two adenomas.

In the third filial generation also there were three liver tumors, all adenomas.

Note that these liver tumors all fell within two lines from this mating, namely, lines A and D. Note also line C, of which 100 per cent shows carcinoma, all of the mammary gland and of the lung; where the parent female had carcinoma of the mammary gland and the parent male had carcinoma of the lung.

*Chart 10*

Branch IX of Strain 338. This branch is derived, of course, from identical ancestry with Branch V, as shown in Chart 9. Here also there are two generations of primary liver tumor.

*Chart 11*

Branches XII and XIII of this same strain show also an outcropping of primary liver tumor in the fourth filial generation, a lymphosarcoma in female No. 12212, and an adenoma in female No. 11245.

*Chart 12*

Parts of Strains 465 and 460. This chart is introduced to show how in hybridization also Strain 338 and its sister strain, Strain 465, produced liver tumor.

Two sisters, female No 5305 and female No. 5417, were mated with two half-brothers, male No. 5215 and male No. 7736. Note the outcropping of liver sarcomas, along with the stomach tumors, in the third and fourth filial generations in each case.

Let me review the striking points demonstrated by these charts:

1. A great many strains have been carried for years in this laboratory yielding varying percentages of cancer, some of them as high as 100 per cent, but never showing liver tumor, primary or secondary. These strains have been carried side by side with the liver tumor strains, have been handled with identical tech-

BRANCH IX

# STRAIN 338

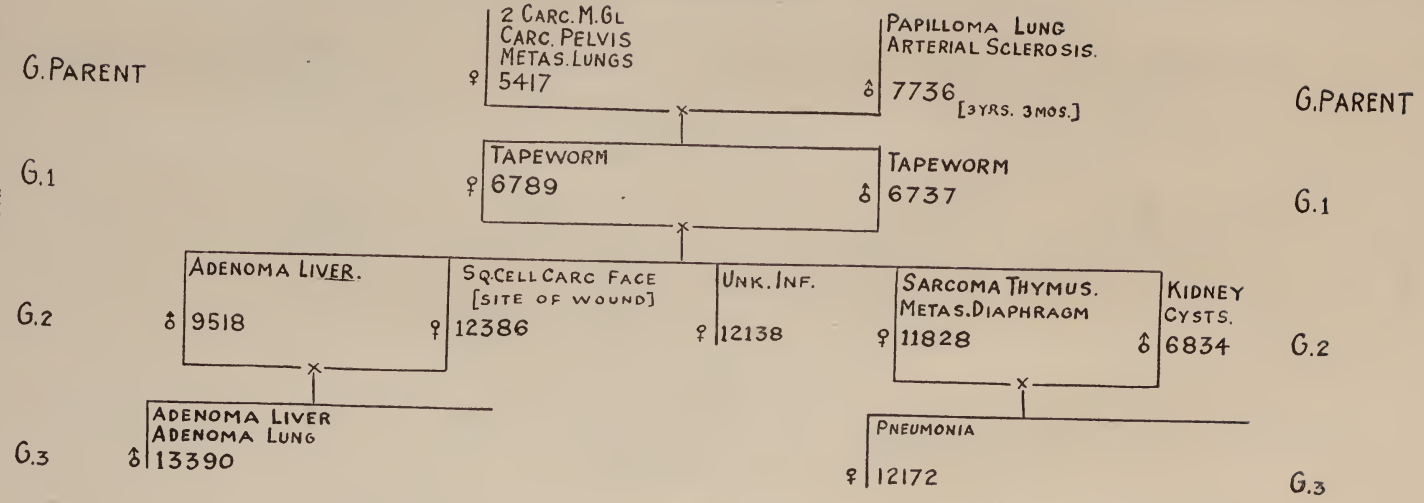



CHART 10

BRANCH XII AND XIII

# STRAIN 338

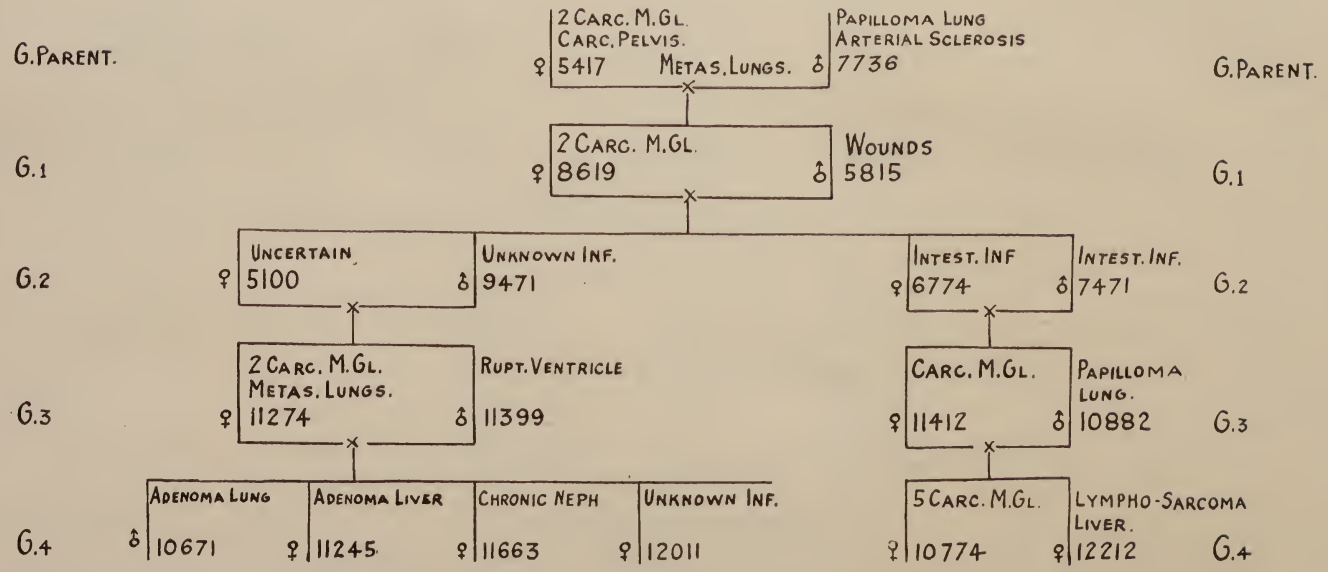



CHART 11

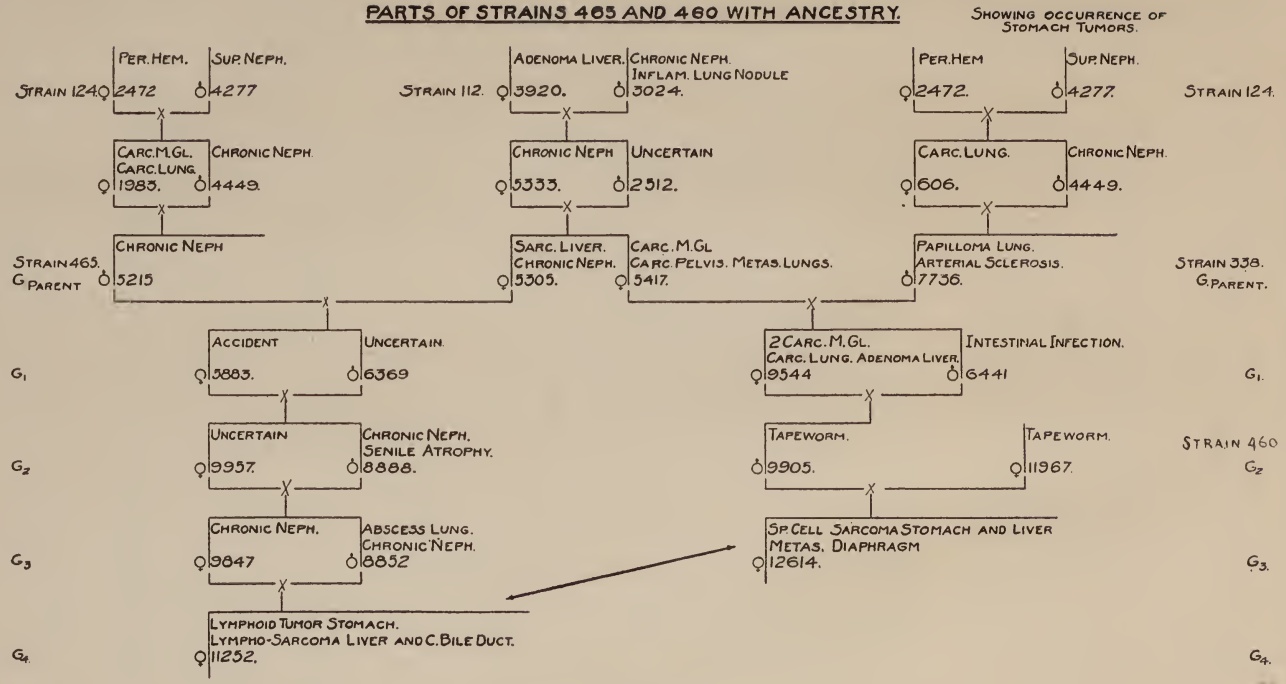

PARTS OF STRAINS 465 AND 460 WITH ANCESTRY.
SHOWING OCCURRENCE OF STOMACH TUMORS.
STRAIN 124
PER. HEM.
SUP. NEPH.
♀2472
♂4277
CARC. M. GL.
CARC. LUNG.
CHRONIC NEPH.
♀1983.
♂4449.
STRAIN 465.
G. PARENT
CHRONIC NEPH
♂5215
STRAIN 112.
ADENOMA LIVER.
CHRONIC NEPH.
INFLAM. LUNG NODULE
♀3920.
♂3024.
CHRONIC NEPH
UNCERTAIN
♀5333.
♂2512.
SARC. LIVER.
CHRONIC NEPH.
CARC. M. GL
CARC. PELVIS. METAS. LUNGS.
♀5305.
♀5417.
PER. HEM
SUP. NEPH.
♀2472.
♂4277.
STRAIN 124.
CARC. LUNG.
CHRONIC NEPH.
♀606.
♂4449.
PAPILLOMA LUNG.
ARTERIAL SCLEROSIS.
♂7756.
STRAIN 338.
G. PARENT.
ACCIDENT
UNCERTAIN.
G1
♀5883.
♂6369
UNCERTAIN
CHRONIC NEPH.
SENILE ATROPHY.
G2
♀9957.
♂8888.
CHRONIC NEPH.
ABSCESS LUNG.
CHRONIC NEPH.
G3
♀9847
♂8852
LYMPHOID TUMOR STOMACH.
LYMPHO-SARCOMA LIVER AND C. BILE DUCT.
G4
♀11252.
2 CARC. M. GL.
CARC. LUNG. ADENOMA LIVER.
INTESTINAL INFECTION.
♀9544
♂6441
G1.
TAPEWORM.
TAPEWORM.
STRAIN 460
♂9905.
♀11967.
G2
SP. CELL SARCOMA STOMACH AND LIVER
METAS. DIAPHRAGM
♀12614.
G3.
G4.

CHART 12

nique, and hybridized with identical non-liver tumor strains, thus eliminating every possible cause for this divergence except heredity.

2. One cancer bearing strain, Strain 90, has been used either in inbreeding or in hybridization (a) in the origin of every strain in this laboratory which has ever produced a primary tumor of the liver; (b) in the origin of every strain which has ever furnished a secondary tumor of the liver, with the exception of two osteosarcomas which metastasized in practically every organ in the body.

3. One individual female, No. 3, with a malignant adenoma of the liver and a sarcoma of the mammary gland, stands out preeminently in this production of liver tumor. Either she or her immediate offspring has been used in one or both origins of every strain which has ever produced a liver tumor, primary or secondary, in this laboratory, with the exception of the two metastatic osteosarcomas noted above.

The emergence of liver tumor in these hybrid strains derived from female No. 3, or her immediate offspring, frequently takes place years after the death of their liver tumor progenitors, thereby eliminating every possibility of a contact transmission of this disease even in the nature of a germ plasm infection.

4. Certain individuals, both when inbred and when outbred and when tested with many mates, consistently show liver tumor in every resulting strain.

Consider (1) that outside of this stock there is just one liver tumor recorded among all the thousands of mouse tumors, (2), that these results are deliberately produced by the manipulation of selective breeding alone, (3) that they have consistently occurred for years in every test made, (4) that there are over twenty-five hundred primary neoplasms, and over fourteen thousand individuals involved in this perfect and persistent consistency. What explanation remains but that of heredity?

# INDEX TO VOLUME I